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THE ADSORPTION OF SOAP BY CARBON BLACK[1]

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Abstract

The adsorption of the sodium soaps of lauric, myristic, palmitic, stearic, and oleic acids from aqueous solutions by a carbon black has been measured. The adsorption appears to be a combination of two more or less independent processes: (*a*) adsorption of neutral soap, and (*b*) adsorption of fatty acid resulting from hydrolysis. The adsorption of both the fatty acid and alkali components of the soaps studied is in agreement with the Freundlich adsorption equation over the concentration range investigated, the extent of adsorption increasing with increasing chain length of the saturated soaps examined. In every case there is a greater adsorption of fatty acid than of alkali, but this difference becomes greater with increasing chain length of the soap. Increase in temperature causes a slight decrease in adsorption, but the effect is small. On the basis of its adsorptive behavior, sodium oleate appears to have an effective chain length of about 15 carbon atoms. Excess of fatty acid in the initial soap solution results in an increased adsorption of total fatty acid, but does not influence the adsorption of neutral soap; on the other hand, excess of alkali in the initial solution not only results in an increased adsorption of total alkali, but also leads to a decrease in the adsorption of fatty acid and neutral soap. This decrease in adsorption is attributed to suppression of hydrolysis, but even with 100% excess alkali, where hydrolysis must be almost completely suppressed, there is still an appreciable adsorption of fatty acid which must be adsorbed in the form of neutral soap. The adsorbed material corresponds to an acid soap of variable composition, the ratio of excess acid to neutral soap depending on the composition of the initial solution, the temperature, and the particular soap used. The adsorption of soap from 95% alcohol solution and from absolute alcohol solution is essentially the same, both being considerably lower than the adsorption of the same soap from aqueous solution. In spite of the fact that hydrolysis of soap does not occur in alcoholic solution, the fatty acid and alkaline components are not adsorbed in equivalent amounts, and it is suggested that, in this case, splitting of the soap is brought about by alcoholysis, followed by preferential adsorption of one of the reaction products.

Introduction

During the course of studies in the field of detergency currently in progress in these laboratories it became of interest to investigate the adsorption of various sodium soaps on carbonaceous materials used as fiber-soiling media. The present paper gives data relating to the adsorption of soaps, or their components, on one carbon which may be regarded as typical of the carbons employed in detergency studies. While these data would not be expected to apply directly to other carbons or complex carbon–oil soils which have been used by other investigators, it is believed that the work is of interest in

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so far as it illustrates the general behavior of soap solutions in contact with finely divided carbonaceous material.

A survey of the literature reveals that only a limited amount of work of a quantitative nature has been reported on the adsorption of soaps by carbon.

Fromageot and Wurmser (2) measured the adsorption of a number of organic acids and their sodium salts in aqueous solution on charcoal and found that the adsorption of the acid was appreciable and was always greater than the adsorption of the salt. The difference between adsorption of acid and of salt varied widely with the various organic radicals; thus the sodium salts of formic, acetic, succinic, and citric acids were not adsorbed at all, whereas the adsorption of sodium pyruvate amounted to more than 80% of that of the free acid. They were unable to find any relation between the dissociation constant of the acid and the adsorption of the salt.

From measurements of the adsorption of various inorganic acids, salts, and bases in aqueous solution on purified low ash charcoals of both animal and vegetable origin, Miller (8) concluded that acids are adsorbed to a considerable extent, bases are not adsorbed at all, and neutral salts are not adsorbed as such, although acid resulting from their hydrolysis is adsorbed.

Mikumo (6) measured the adsorption of potassium oleate in aqueous solution on a variety of adsorbents and concluded that carbon (Merck's animal charcoal) adsorbs a complex soap, which always contains an excess of fatty acid, even from initially alkaline solutions. He points out that the presence of carbon may thus greatly promote the hydrolysis of soap owing to displacement of the hydrolysis equilibrium.

Mikumo (7) also measured the adsorption of a series of saturated potassium soaps in absolute alcohol solution on charcoal (Merck's animal charcoal purified with alcohol and ether), and found that the adsorption was in agreement with the Freundlich equation (1) up to the "concentration of aggregate-formation", the extent of adsorption increasing with increasing molecular weight of the soap.

Neville and Harris (9) measured the pH of an aqueous solution of an olive oil soap after agitation with lampblack and filtering, and found that the filtrate was more alkaline than the original solution. They attributed this increased alkalinity to the selective adsorption of either free fatty acid or acid soap by the lampblack.

Experimental

Materials and Methods

The adsorbent used throughout these experiments was uncompressed standard Micronex,* a channel black having a mean particle diameter of about 28mμ.

* *Supplied by Binney and Smith Co., New York.*

Sodium soaps of lauric, myristic, palmitic, stearic, and oleic acids were prepared from Kahlbaum or Schuchardt fatty acids following the method described by Powney (10). The melting point, acid number, and "percent purity" calculated from the acid number of the fatty acids are listed in Table I. The alcohol used in the preparation of the soaps was freed from aldehydes by refluxing with powdered zinc and potassium hydroxide, followed by distillation.

TABLE I

CHARACTERISTICS OF FATTY ACIDS USED IN PREPARATION OF SOAPS

Acid	Melting point, °C.	Acid No. Mgm. KOH/gm. acid	Purity, %
Lauric	44.0	281.5	100.5
Myristic	53.0	244.0	99.3
Palmitic	62.5	216.5	98.8
Stearic	69.0	196.5	99.4
Oleic	——	193.5	97.4

Distilled water was used in the preparation of all aqueous solutions.

The adsorption of the fatty acid and alkali components of the various soaps was determined by the following method except where otherwise noted.

A 1.000 gm. portion of carbon black was weighed into a previously warmed 400 ml. vacuum bottle, and agitated for 10 min. with 250 ml. of soap solution having the desired temperature and initial concentration, using a mechanical shaking device. After shaking, the suspension was weighed, and filtered under suction through three thicknesses of Whatman No. 42 filter paper supported in a covered, electrically heated Büchner funnel. The filter cake and filtrate were each weighed, and any loss in weight (due to evaporation of water during filtration) was made up by the addition of distilled water to the filtrate.

A 100 ml. aliquot of the filtrate was taken for analysis and a blank determination was also carried out on 100 ml. of the original soap solution filtered in the same way. The soap solution was acidified with an excess of $N/100$ sulphuric acid, heated almost to boiling, and cooled. The liberated fatty acid was extracted with three successive portions of diethyl ether which had been previously rendered neutral by distillation over sodium hydroxide. The combined ether extracts were washed once with water, the washings being added to the aqueous residue from the extractions. The aqueous portion was boiled gently to expel dissolved ether and titrated with $N/100$ sodium hydroxide and phenolphthalein. The ether was removed from the extracted fatty acid by distillation, followed by warming on a boiling water bath. The residue was then redissolved in neutral 95% ethyl alcohol and titrated hot with $N/100$ sodium hydroxide and phenolphthalein.

The adsorption of either fatty acid or alkali is given by the formula $x = 0.25 (C_0 - C)$, where x is the quantity adsorbed in milliequivalents per gram of carbon, and C_0 and C are the concentrations of the reference and equilibrium solutions respectively in milliequivalents per liter ($M \times 10^{-3}$).

Where percentage concentrations of fatty acid or alkali are used, these are expressed in terms of the equivalent weight of sodium soap.

Preliminary Experiments

A series of preliminary experiments was carried out to determine the extent of experimental error and hence to evaluate the probable accuracy of the method.

Carbon.—A 0.4% suspension of carbon in distilled water at 70°C. was shaken for 10 min. and then filtered. No titratable amount of acid or of alkali was found in the filtrate. A similar test was carried out using 95% ethyl alcohol at 25°C. in place of the water. Again no measurable amount of acid or alkali was extracted from the carbon.

Tin foil.—It was observed that the pure tin foil used to cover the stoppers of the vacuum bottles became dull after continued use, particularly with solutions containing excess alkali. In order to determine the effect on the adsorption values of any possible reaction with the tin, pieces of foil having an area equal to 6 to 8 times that normally in contact with the solution were shaken with 250 ml. of 0.07% sodium hydroxide solution at 70°C. for 30 min. There was no detectable change in the concentration of the sodium hydroxide solution at the end of this time.

Adsorption on filter paper.—Errors due to adsorption of soap by the filter paper during the filtration operation were investigated. It was found that the decrease in concentration of a 0.1% solution of sodium stearate after filtering was less than 1%. This is considered negligible and, in any case, is compensated for by filtration of the reference solution prior to analysis.

Rate of adsorption.—The adsorption of fatty acid and alkali from a 0.1% solution of sodium stearate was determined for a range of shaking times varying from 5 to 80 min. It was found that equilibrium was established within the first five minutes and that no further adsorption took place on longer shaking. Nevertheless, in order to allow an adequate margin of safety, a shaking time of 10 min. was adopted for all subsequent work.

Over-all errors.—Errors arising from all sources, which may include mechanical losses in handling, inaccuracy in measurement, effect of atmospheric carbon dioxide, and adsorption by glass and filter paper, were evaluated by working through the complete procedure using a 0.1% solution of sodium stearate, but omitting the carbon. The apparent adsorption obtained was 0.017 and 0.021 milliequivalents per gm. for fatty acid and alkali respectively. This represents an error in the adsorption data for 0.1% sodium stearate of 2.4 to 4.3%.

Effect of Soap Concentration on Adsorption

The adsorption of fatty acid and of alkali from aqueous solutions of sodium laurate and sodium stearate of initial concentration varying from 0.01 to 0.20% was measured at 70°C. The results are given in Fig. 1.

The Freundlich adsorption equation (1) reduced to its simplest form may be written $x = kC^n$ or $\log x = \log k + n \log C$, where x is the amount of material adsorbed (millimoles per gram of carbon), C is the equilibrium concentration ($M \times 10^{-3}$), and k and n are experimentally determined constants. Since a plot of $\log x$ vs. $\log C$ should give a straight line, it is apparent that the adsorption of both the fatty acid and alkali components of these soaps over the concentration range investigated is in agreement with the Freundlich equation.

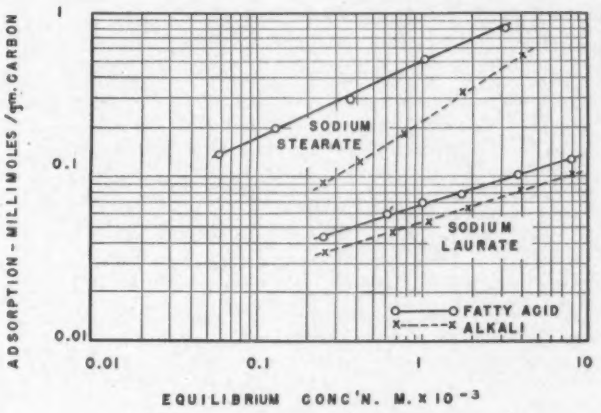

FIG. 1. *Adsorption of soap components.*

The constant k is a measure of the relative magnitude of the adsorption at a fixed equilibrium concentration, i.e., in the present case k represents the adsorption in millimoles per gram of carbon when the equilibrium concentration is $1 \times 10^{-3}M$. The constant n is a measure of the rate at which adsorption increases with increasing equilibrium concentration. The values of these constants (Table II) thus describe completely the adsorption of each component of the soaps for the particular adsorbing material and solvent used, and for the concentration range over which they are applicable.

There is a greater adsorption of fatty acid than of alkali from the initially neutral* soap solution. This is in agreement with the findings of Mikumo (6) for potassium oleate, and also accounts for the increased alkalinity of soap solutions after contact with carbon noted by Neville and Harris (9).

* *The term "neutral" soap is used to indicate equivalence of the fatty acid and alkali constituents of the soap. The aqueous solution is, of course, alkaline in reaction owing to hydrolysis of the soap.*

TABLE II

VALUES OF THE ADSORPTION CONSTANTS

Adsorbate	Component	Solvent	Equilibrium conc. range $M \times 10^{-3}$	k	n
Sod. laurate	Fatty acid	Water	0.25 - 8.0	0.068	0.31
Sod. laurate	Alkali	Water	0.25 - 8.0	0.053	0.31
Sod. stearate	Fatty acid	Water	0.06 - 3.2	0.495	0.45
Sod. stearate	Alkali	Water	0.25 - 4.0	0.216	0.64
Sod. hydroxide	——	Water	0.68 - 23.4	0.140	0.29
Lauric acid	——	Alcohol	2.3 - 22.0	0.0095	0.93
Stearic acid	——	Alcohol	0.9 - 13.0	0.038	0.49

Effect of Chain Length of Soap on Adsorption

The adsorption of fatty acid and of alkali from soap solutions of initial concentration 0.1% was determined at 70°C. for the sodium soaps of lauric, myristic, palmitic, stearic, and oleic acids. The results are given in Fig. 2.

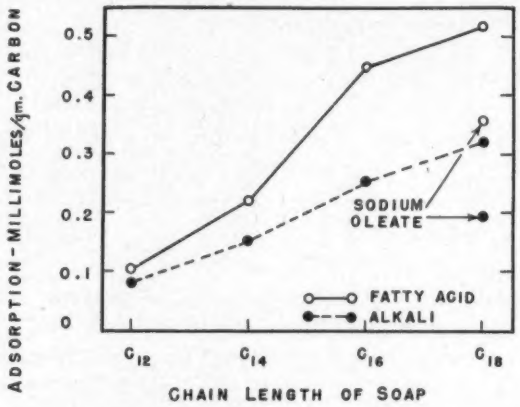

FIG. 2. *Effect of chain length of soap on adsorption.*

It will be observed that there is an increase in adsorption of both fatty acid and alkali with increasing chain length of the saturated soaps. There is also a greater adsorption of fatty acid than of alkali in every case, but this difference is greater for the longer chain soaps than for the shorter.

It is well known that the presence of the double bond in sodium oleate results in a shortening of the effective chain length. This is confirmed by the present data since the adsorption of both fatty acid and alkali from sodium oleate solutions lies between the corresponding adsorptions from sodium myristate and sodium palmitate. On the basis of these data the effective chain length of sodium oleate is approximately 15 carbon atoms.

Effect of Temperature on Adsorption

The effect of temperature on adsorption at 0.1% initial concentration was determined for the sodium soaps of lauric, stearic, and oleic acids (Table III). There appears to be a slight decrease in adsorption of both components of the soaps with increasing temperature, but the effect of temperature is small and in most cases scarcely greater than the probable error involved in its determination.

TABLE III

EFFECT OF TEMPERATURE ON ADSORPTION

Soap	Component	Adsorption, millimoles/gm. carbon			
		30°C.	50°C.	70°C.	80°C.
Sod. laurate	Fatty acid	0.104	0.110	0.104	
Sod. laurate	Alkali	0.093	0.096	0.080	
Sod. stearate	Fatty acid			0.513	0.488
Sod. stearate	Alkali			0.321	0.307
Sod. oleate	Fatty acid	0.382	0.372	0.355	
Sod. oleate	Alkali	0.222	0.202	0.192	

*Adsorption of Alkali**

The adsorption of sodium hydroxide from aqueous solution at 70°C. was measured at initial concentrations varying from 0.005 to 0.1%. The method was the same as was used for the determination of alkali adsorption from soap solutions, except that the ether extraction was omitted. It will be seen (Fig. 3

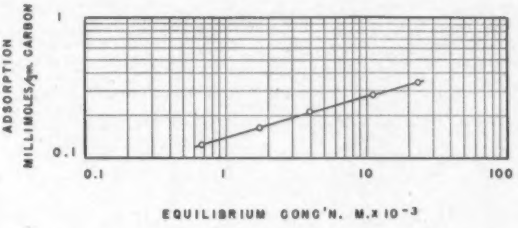

FIG. 3. *Adsorption of sodium hydroxide.*

and Table II) that the adsorption of sodium hydroxide is in agreement with the Freundlich equation over the concentration range studied. This is in contrast to the findings of Miller (8) who reported no adsorption of sodium hydroxide from aqueous solutions by purified charcoal.

The disagreement between the present data and those of Miller can probably be attributed to the presence of materials other than chemically pure carbon contained in the carbon black. Miller has suggested that the discordant data

* *A second batch of standard Micronex was used for this and subsequent work. Adsorption values obtained with this material were somewhat higher than those obtained with the previously used material.*

relative to the adsorption of alkali by carbon reported in the literature prior to the time of his writing are due to the presence of adsorbed acids on the carbon, and that the observed reduction in concentration of the solution after contact with carbon is due to neutralization of these acids, rather than to true adsorption.

However, since no attempt was made to purify the carbon black used in the present work, this material cannot be compared to the highly purified charcoals used by Miller. While part of the observed consumption of alkali may possibly be due to neutralization of small amounts of acid contained in the carbon, the slope of the curve in Fig. 3 indicates that adsorption of hydroxide by some constituent of the carbon black has taken place. Had the consumption of alkali been due entirely to neutralization, the curve obtained would have been a straight line parallel to the concentration axis.

This view was confirmed by attempts to measure the amount of acid contained in the carbon by the method given by Miller (8), in which a weighed amount of carbon is boiled with a known volume of standard sodium hydroxide solution, filtered, washed thoroughly, and the hydroxide in the filtrate titrated with standard acid. It was found that the value for the amount of acid contained in the carbon increased with increasing concentration of the sodium hydroxide solution used. This clearly indicates that the removal of hydroxide from solution is due, in part at least, to adsorption.

Adsorption of "Unbalanced" Soaps

The term "unbalanced" is used to describe solutions in which the fatty acid and alkali components of the soap are not present in equivalent proportions. For this work, batches of sodium laurate and sodium stearate were prepared containing approximately 15% excess fatty acid, i.e., in the preparation of the soap only 85% of the fatty acid was neutralized with sodium hydroxide. Solutions containing lower amounts of excess fatty acid and varying amounts of excess alkali were prepared by adding to a solution of the above "acid" soap the required amount of sodium hydroxide solution.

Solutions containing any appreciable excess of fatty acid were quite cloudy owing to the presence of suspended fatty acid or acid soap, and on filtering, it was found that a small amount of the fatty acid (approximately 2% of the excess) was retained by the filter paper. This would lead to some error in determining the concentration C_0 of the reference solution. On the other hand, filtrates from these solutions after shaking with carbon were perfectly clear, and were found to contain an excess of alkali, indicating that all of the excess fatty acid had been removed by the carbon. The loss on filtration of such solutions was shown previously to be negligible. In view of these considerations, filtration of the reference solution was eliminated throughout this section of the work.

The adsorption of fatty acid and alkali from sodium laurate and sodium stearate solutions at 70°C. was measured for solutions containing initially

0.1% of neutral soap and varying amounts of excess fatty acid or of alkali. The results are given in Fig. 4.

In comparing the curves for fatty acid and alkali adsorption, it may be assumed that the lower of the two curves at any point on the abscissa represents the adsorption of neutral soap (or at least the adsorption of fatty acid and alkali in equivalent proportions), while the difference between the two curves represents the preferential adsorption of either fatty acid or alkali, depending on the relative positions of the two curves. On this basis it may be concluded that the adsorption of neutral soap is not influenced by the presence of varying amounts of excess fatty acid. It is probable that only the fatty acid actually in solution can influence the adsorption of neutral soap, and once the solubility limit has been reached, further additions of fatty acid have no effect. On the

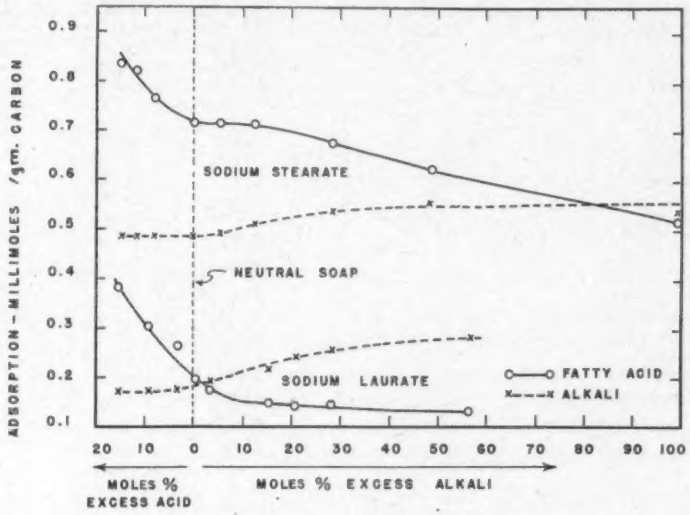

FIG. 4. *Adsorption of components of unbalanced soaps.*

other hand, the suspended fatty acid (or acid soap) is readily adsorbed by the carbon, as is shown by the rapid increase in adsorption with increasing amounts of excess fatty acid.

In the presence of increasing amounts of excess alkali, the alkali adsorption increases gradually while the fatty acid adsorption tends to decrease. In the case of sodium laurate the two curves cross at about 2 mole % excess alkali, but with sodium stearate a preferential adsorption of fatty acid is indicated until about 80 mole % excess alkali is reached.

Adsorption from Alcoholic Solution

In these experiments the soap was dissolved in freshly distilled 95% ethyl alcohol. The adsorption determinations were carried out as for aqueous

solutions except that after measurement of the aliquot of filtrate the alcohol was distilled off and was replaced by an equal volume of distilled water. The adsorption of sodium laurate at 0.1% initial concentration and of sodium stearate at 0.05 and 0.1% was determined at 25°C. A similar series of experiments was carried out in which absolute alcohol and dried reagents were used, and exposure to atmospheric moisture was limited to the filtration period. The results are given in Table IV, in which the corresponding values for aqueous solutions are also included.

TABLE IV

COMPARISON OF ADSORPTION OF SOAP FROM AQUEOUS AND ALCOHOLIC SOLUTION

Soap	Component	Initial conc., %	Adsorption, millimoles/gm. carbon		
			Aqueous solution	95% alcohol solution	Absolute alcohol solution
Sod. laurate	Fatty acid	0.1	0.195	0.041	
Sod. laurate	Alkali	0.1	0.182	0.135	
Sod. stearate	Fatty acid	0.05		0.025	0.026
Sod. stearate	Alkali	0.05		0.085	0.087
Sod. stearate	Fatty acid	0.1	0.719	0.036	0.032
Sod. stearate	Alkali	0.1	0.485	0.097	0.103

The data show that the adsorption of both fatty acid and alkali is considerably lower from alcoholic solution than from aqueous solution, particularly in the case of sodium stearate. Furthermore, the preferential adsorption of fatty acid observed with aqueous solutions does not occur with alcoholic solutions, there being, on the contrary, a preferential adsorption of alkali in the latter case.

The results obtained with absolute alcohol are not significantly different from those obtained with 95% alcohol, indicating that the presence of small amounts of water has no measurable effect on the adsorption.

The adsorption of free lauric and stearic acids from 95% alcohol solutions of varying initial concentrations was also determined at 25°C., the data being given in Table II and Fig. 5.

Discussion

Two possible mechanisms for the adsorption of soap from aqueous solution by carbon may be postulated, viz.: (*a*) neutral soap is not adsorbed as such, but the products of hydrolysis are adsorbed [this view is expressed by Miller (8) in relation to the adsorption of inorganic salts]; (*b*) neutral soap is adsorbed as such and, in addition, the hydrolytic products are also adsorbed, each adsorption taking place more or less independently.

If only the hydrolytic products are adsorbed, suppression of hydrolysis should lead to a marked reduction in adsorption. It was hoped that measurements of the adsorption of soap from alcoholic solutions might throw some light on this point since hydrolysis is completely suppressed in solutions containing 40% or more of ethyl alcohol (3, 11, 12). While it is true that a marked reduction in the adsorption of soap occurred when alcohol was used in place of water, it was found that the adsorption of free fatty acid from alcoholic solution was also very low (Fig. 5). It is probable, therefore, that the decreased adsorption of soap from alcoholic solutions as compared to that from aqueous solutions is not due entirely to the suppression of hydrolysis by alcohol.

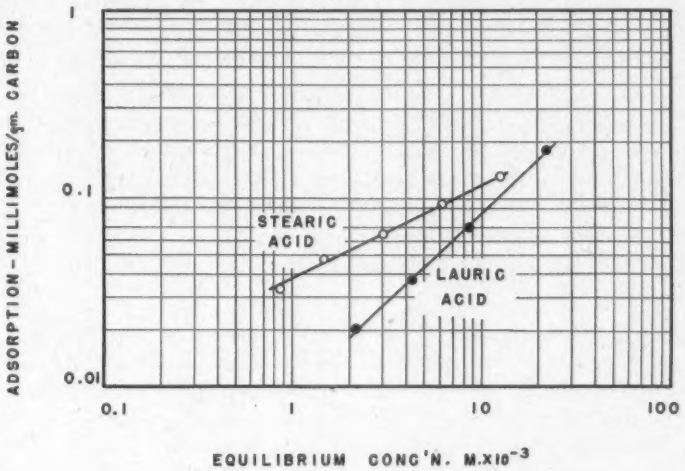

FIG. 5. *Adsorption of fatty acids from alcohol solution.*

From adsorption measurements in aqueous solution (Fig. 4) it is apparent that an excess of free alkali causes a decrease in the adsorption of fatty acid. McBain and Martin (5) have concluded that the presence of 3 to 4% excess sodium hydroxide is sufficient to suppress completely the hydrolysis of sodium palmitate in 0.1% aqueous solution at 90°C. It is reasonable to assume that the decrease in fatty acid adsorption observed is due in part at least to the suppression of hydrolysis by the excess alkali, since neutral soap is probably adsorbed less readily than is the hydrolytic fatty acid (2). On the other hand, even in the presence of 100 moles % excess alkali there is still an appreciable adsorption of fatty acid, and it must therefore be concluded that this fatty acid is adsorbed in the form of neutral soap molecules. This indicates that the mechanism of adsorption from aqueous solution is a combination of adsorption of neutral soap and hydrolytic fatty acid. In the presence of increasing amounts of excess fatty acid the adsorption of neutral soap remains essentially constant whereas the adsorption of fatty acid increases rapidly

(Fig. 4). This would indicate that the adsorptions of the two species take place more or less independently of each other.

From measurements in alcoholic solutions Mikumo (7) found that the value of the constant n in the Freundlich equation was essentially constant for all soaps examined. On the other hand, the present data for aqueous solutions (Table II) indicate that the value of n varies with different soaps. The difference in the two cases may be attributed to the effect of hydrolysis. In the case of alcoholic solution, since hydrolysis is suppressed, the value of n is determined only by the adsorption behavior of the neutral soap. With aqueous solution the value of n for the fatty acid adsorption curve is dependent on the adsorptive characteristics of neutral soap, and also on those of the hydrolytic fatty acid. Since the degree of hydrolysis varies with different soaps, it is to be expected that the value of n will also vary.

Further confirmation of this view is to be found in the data shown in Fig. 2. If the alkali adsorption curve in this figure is taken to represent the adsorption of neutral soap and the difference between the two curves to represent the preferential adsorption of fatty acid, then it follows that not only does the total adsorption of fatty acid and alkali increase with increasing chain length, but the difference between the two, representing the adsorption of hydrolytic fatty acid, also increases with increasing chain length, i.e., with increasing degree of hydrolysis.

It is thus to be concluded that the second of the two mechanisms postulated is the correct one. The carbon particle with its adsorbed soap film may be likened to a micelle of acid soap of variable composition having a carbon particle as a nucleus. Whether this process is considered as adsorption of soap by the carbon or as "solubilization" of carbon by the soap would seem to be dependent only on the relative amounts of the two substances being considered.

Referring again to the adsorption measurements in alcoholic solution, it will be seen that the total adsorption is relatively low, and that there is a small but significant preferential adsorption of alkali. This suggests that some splitting of the soap molecule into its component parts must occur even in absolute alcohol solution. Theoretically this might be due to simple hydrolysis since traces of moisture were undoubtedly present in spite of attempts to maintain the system in as dry a condition as possible. However, hydrolysis is believed to be suppressed completely in alcohol solution and this claim is confirmed by the fact that adsorption values obtained using 95% and absolute alcohols as solvents were not significantly different. If the splitting of the soap molecule is to be attributed to reaction with water, the adsorption figures for solutions containing 5% of water should be noticeably different from those for solutions containing only a trace of water.

The other alternative is reaction with the alcohol itself, viz., alcoholysis. Such a reaction would have to take place to only a slight extent in order to

account for the experimental data, since the equilibrium would be displaced owing to adsorption of the reaction products. In order to explain the experimental data it would then be necessary to assume that sodium hydroxide is more readily adsorbed than either sodium stearate or ethyl stearate, from alcoholic solution.

From the data for fatty acid and alkali adsorption it is possible to calculate the composition of the adsorbed material in terms of the molar ratio of excess fatty acid to neutral soap (the neutral soap term may include, in addition to neutral soap, free fatty acid and sodium hydroxide, products of hydrolysis, in equivalent amounts). The effects of initial composition of the soap solution, chain length of the soap, and temperature, on the composition of the adsorbed

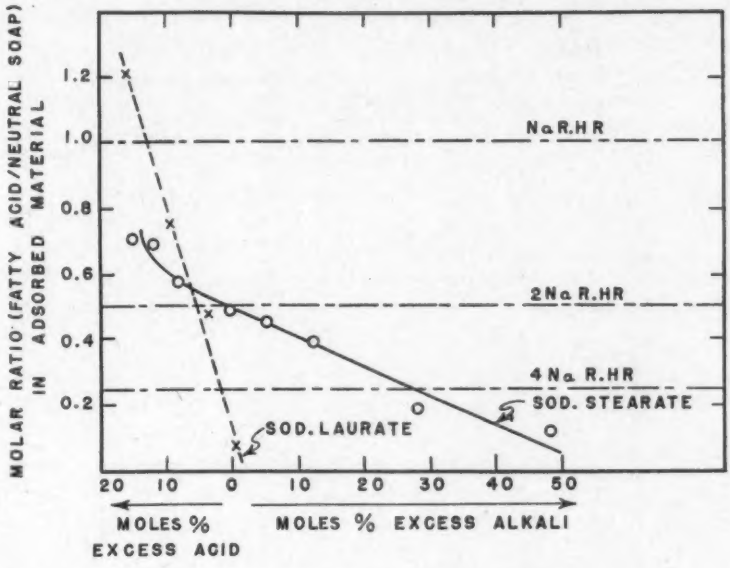

FIG. 6. *Composition of adsorbed material.*

material are shown in Figs. 6, 7, and 8. From these data there appears to be no indication of the formation or adsorption of acid soaps of a constant composition. It is interesting to note (Fig. 6) that the addition of lauric acid to sodium laurate, which is a slightly hydrolyzed soap, causes a rapid change in the composition of the adsorbed material. On the other hand, the addition of stearic acid to sodium stearate causes a much smaller change in the composition of the adsorbed material. Sodium stearate is strongly hydrolyzed; consequently there is a considerable amount of hydrolytic fatty acid present in the initial solution and the addition of more fatty acid has much less effect than with sodium laurate, where the amount of hydrolytic fatty acid is low.

The effect of temperature on the composition of the adsorbed material (Fig. 7) can be explained on the basis of hydrolysis, since the degree of hydrolysis increases with increasing temperature (4).

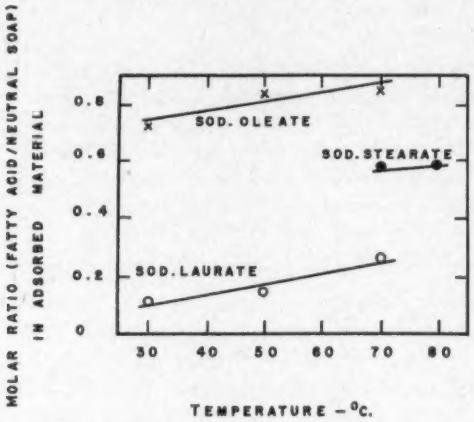

FIG. 7. *Effect of temperature on composition of adsorbed material.*

The data given in Fig. 8 may also be explained on the basis of hydrolysis. In general the degree of hydrolysis increases with increasing chain length of the soap, and consequently adsorbed films of the higher soaps contain a greater

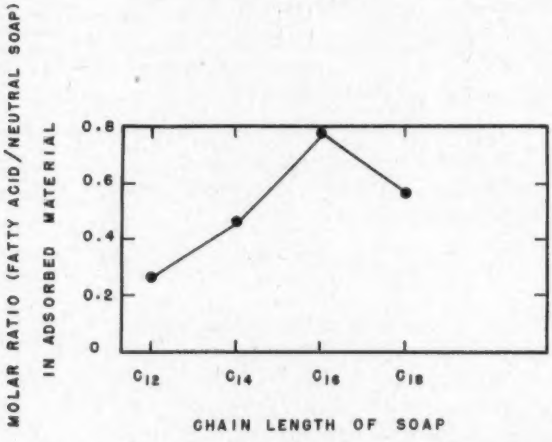

FIG. 8. *Effect of chain length of soap on composition of adsorbed material.*

proportion of free fatty acid than do those of the lower soaps. The explanation of the apparent discrepancy in the palmitate and/or stearate values is probably to be found in the hydrolytic behavior of these soaps. Under certain conditions

sodium palmitate may be more strongly hydrolyzed than sodium stearate, e.g., at 25°C. a 0.002 *N* solution of sodium palmitate is 15.8% hydrolyzed, whereas sodium stearate solution of the same concentration is only 10.0% hydrolyzed (4). While data are not available for the hydrolysis of these soaps at 70°C., it is conceivable that at the particular temperature and concentration employed the hydrolysis of sodium palmitate may exceed that of sodium stearate.

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COLLABORATIVE ANALYSIS OF WHEAT, OATS, AND BARLEY FOR THIAMINE AND RIBOFLAVIN[1]

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Abstract

In the analysis of wheat, oats, and barley for thiamine and riboflavin by three collaborating laboratories an attempt was made to reduce interlaboratory differences to a minimum by the establishment of a standardized procedure. The thiochrome and fluorometric methods were used for the thiamine and riboflavin determinations respectively. It was apparent that even after steps had been taken to standardize the assay procedures, small but consistent and significant interlaboratory errors existed. Calculations indicate that differences of the order of 10 to 12% in mean assay values were necessary before samples could be considered different with respect to their thiamine or riboflavin content.

Introduction

Experience has shown that different laboratories, analyzing the same samples of biological material for thiamine and riboflavin, may get results showing a wide variation. Thus, reports of the National Check Sample Committee of the American Association of Cereal Chemists (1, 4, 5) show that the mean values found by the co-operating laboratories ranged from 5.28 to 8.26 μgm. per gm. for thiamine in the six 1943-44 check samples, from 4.28 to 5.37 μgm. per gm. for six 1944-45 check samples, and from 3.09 to 5.31 μgm. per gm. for six 1946-47 samples. The results for riboflavin in the 1946-47 samples ranged from 1.77 to 3.76 μgm. per gm.

Obviously when such variations are possible, results from different laboratories cannot be readily compared. The laboratories of the Canadian prairie universities were conducting vitamin surveys of grains grown in their respective provinces, and it was desirable that the results should be comparable. It was thought that if the same methods were used in different laboratories, and if each step in the two procedures was standardized rigidly, it might be possible to obtain results agreeing much more closely. Accordingly, the three co-operating laboratories initiated the study, the results of which are reported herein.

Methods and Materials

The method adopted for the thiamine assay was essentially the thiochrome procedure as outlined by Hennessy (3). Incubation with takadiastase followed the acid digestion. The initial digestions were done in 100 ml. volumetric

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flasks to avoid possible loss in transferring the mixtures. Greater ease and uniformity of percolation were obtained when the adsorption columns were prepared by pouring Decalso into exchange tubes which had just been filled with water. The thiamine was removed from the Decalso by eluting with hot potassium chloride solution. The oxidation of the thiamine to thiochrome with alkaline ferricyanide was facilitated by injection of the oxidizing agent into the thiamine eluate by means of a hypodermic syringe.

Chemicals with low fluorescence were used to keep blank readings at a minimum. Several lots of anhydrous sodium sulphate were rejected for this reason. Initially, aliquots from the same batch of Decalso and quinine sulphate were used by the co-operating laboratories. New lots of chemicals were checked with the ones formerly used.

Calculations were made by comparing the sample readings with those for external standards, that is, an aliquot of pure thiamine hydrochloride solution, subjected to oxidation and extraction with isobutanol, but not to the earlier steps of the procedure. These standards were tested daily, with the weekly average being used for the final calculations. Duplicates were done on successive days. Assays which differed by more than 5% from the mean of that sample were repeated. Since exhaustive tests revealed that recovery of thiamine from samples using this procedure is only 94%, in an external standard, the results were therefore adjusted for 94% recovery. Each laboratory used a Coleman Electronic Photofluorometer, Model 12, for measurements. Readings of standard solutions were checked between laboratories.

The method adopted for the assay of riboflavin was essentially the fluorometric one reported by Andrews (2). Initial acid digestion was followed by takadiastase hydrolysis. Further purification was obtained by adsorption of the riboflavin on Florisil. The preparation of the adsorption columns was modified to the extent that a hot water suspension of Florisil was added to the exchange tubes. A measured volume—0.25 ml.—of ice-cold solution of sodium hydrosulphite and sodium bicarbonate was used instead of sodium hydrosulphite crystals. An external standard of riboflavin in pyridine – acetic acid solution was used for comparison, rather than one obtained by adding riboflavin to the sample. Duplicates were done on successive days. A slightly greater margin of error was allowed than was permitted for thiamine since the riboflavin content of cereals is considerably smaller.

In order to standardize a method for the three laboratories common samples of wheat, oats, and barley of the 1945 crop were used. These were ground in one of the laboratories and aliquots distributed. The collaborative method for thiamine and riboflavin was then employed to assay 59 wheat, 47 oat, and 46 barley samples of the 1946 crop which had been grown in the three provinces. These samples were obtained from University test plots, Experimental Farm and Illustration station plots as well as from Junior Grain Club plots, and were pure strains. They were widely distributed, being grown on dark brown,

black, light brown, and gray soil types. The samples were ground in the province of origin and portions distributed to the other two laboratories. Moisture was determined by the 130°C. air oven method.

Results and Discussion

Results are reported in micrograms per gram at a 13.5% moisture level. The mean values shown in Table I, obtained from the three laboratories, indicate the following range observed in thiamine and riboflavin content of samples of wheat, oats, and barley grown in different locations in the prairie provinces. There appears to be a high correlation between the protein and thiamine content. This is borne out by results from one of us (E.Y.S.) on a much larger number of wheat samples. Correlations in this last survey are also being made between thiamine and variety and soil type.

TABLE I

RANGE OF THIAMINE AND RIBOFLAVIN CONTENT OF CEREALS

Cereal	No. of samples	Thiamine, μgm./gm.	% Protein*	Riboflavin, μgm./gm.
Wheat	59	3.48 – 6.35	8.7 – 16.9	0.94 – 1.47
Oats	46	4.75 – 9.35	10.3 – 15.9	0.99 – 1.61
Barley	47	2.89 – 6.32	8.7 – 14.9	0.96 – 1.63

* *Protein values are for the respective samples containing the least and most thiamine.*

Table II indicates the mean, mean deviation, and standard deviation within laboratories and sum of differences from the mean of the three laboratories for the three cereals.

An analysis of variance for the thiamine and riboflavin data from the three laboratories is shown in Table III.

This analysis shows that, with the exception of thiamine in oats, the F values are highly significant for variance attributable to the results obtained at different laboratories.

Minimum significant sample and interlaboratory differences between samples and laboratories were calculated at the 5% level and are included in Table IV.

It was found that, for thiamine in wheat and barley, differences in the results obtained in laboratories B and C were not statistically significant, while those obtained at laboratory A were significantly lower. In the case of riboflavin, the results from laboratory A were significantly lower for wheat and oats, while, for riboflavin in barley, the results from laboratory C were significantly higher than those from the other two. It is thus apparent that even after steps were taken to standardize the assay procedures and, as far as possible, the equipment in the collaborating laboratories, small but consistent and significant interlaboratory errors existed. As indicated above and in Table II, this was

TABLE II

SUMMARY OF VITAMIN ASSAYS

μgm./gm.

	Thiamine assays				Riboflavin assays			
	Lab. *A*	Lab. *B*	Lab. *C*	Three labs.	Lab. *A*	Lab. *B*	Lab. *C*	Three labs.
Wheat								
Mean	4.41	4.79	4.73	4.64	1.08	1.17	1.15	1.13
Mean deviation	0.08	0.13	0.14		0.03	0.04	0.02	
St. deviation	0.44	0.54	0.56	0.57	0.08	0.15	0.11	0.15
Sum of differences from mean	−13.82	8.58	5.18		−3.35	2.49	0.95	
Oats								
Mean	6.86	6.86	6.95	6.89	1.27	1.32	1.33	1.31
Mean deviation	0.10	0.12	0.12		0.04	0.05	0.03	
St. deviation	1.45	1.28	1.36	1.37	0.18	0.12	0.19	0.14
Sum of differences from mean	−1.51	−1.21	2.77		−1.78	0.54	1.14	
Barley								
Mean	4.34	4.50	4.59	4.47	1.27	1.25	1.32	1.28
Mean deviation	0.08	0.12	0.10		0.04	0.05	0.02	
St. deviation	0.60	0.71	0.75	0.74	0.22	0.16	0.22	0.19
Sum of differences from mean	−7.00	1.55	5.55		−0.39	−1.55	1.94	

TABLE III

ANALYSIS OF VARIANCE

Variance due to	Thiamine		Riboflavin	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Wheat				
Labs	2	5.01**	2	0.30**
Samples	58	1.29**	59	0.05**
Labs × samples	116	0.17**	118	0.013**
Error	177	0.052	180	0.004
Total	353		359	
Barley				
Labs	2	1.64**	2	0.13**
Samples	46	2.92**	46	0.20**
Labs × samples	92	0.11**	92	0.011**
Error	141	0.041	141	0.005
Total	281		281	
Oats				
Labs	2	0.58	2	0.10**
Samples	45	10.99**	45	0.15**
Labs × samples	90	0.31**	90	0.02**
Error	138	0.047	138	0.005
Total	275		275	

** *Significant beyond the 1% level.*

due largely to the fact that assay values obtained at one laboratory were, with the exception of those for thiamine in oats and riboflavin in barley, consistently lower than those obtained at the other two laboratories.

TABLE IV

MINIMUM SIGNIFICANT DIFFERENCES

	Thiamine			Riboflavin		
	Samples		Laboratories, μgm./gm.	Samples		Laboratories, μgm./gm.
	μgm./gm.	%		μgm./gm.	%	
Wheat	0.48	10.3	0.11	0.13	11.5	0.03
Oats	0.38	8.5	0.16	0.16	12.3	0.04
Barley	0.64	9.2	0.10	0.12	9.4	0.03

It is shown in Table III that the F values for variance due to samples were highly significant even though the variance for the interaction between laboratories and samples was used as error. This was anticipated, since the samples varied with respect to origin and variety.

Thus the results of this study, in which duplicate assays were done on successive days in three collaborating laboratories, show that after taking into account intralaboratory error and the interaction between laboratories and samples, differences of 8.5 to 10.3% and 9.4 to 12.3% were necessary before samples could be considered different with respect to content of thiamine or riboflavin respectively.

A direct comparison between results cited (1, 4, 5) and those reported here is difficult. The large range in thiamine content shown in Table I contributes a disproportional amount to the magnitude of the standard deviation so that the coefficient of variability as used in the 1946-47 report of the Check Sample Committee (1) cannot be employed for comparative purposes as a measure of precision. However, examination of the minimum significant differences in Table IV indicates that an improvement in precision by collaborating groups has been achieved.

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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

XXXI. PILOT PLANT STUDIES ON THE FERMENTATION OF WHEAT BY *AEROBACILLUS POLYMYXA*[1]

By A. C. Blackwood,[2] J. A. Wheat,[3] J. D. Leslie,[3]
G. A. Ledingham,[4] and F. J. Simpson[2]

Abstract

In this paper are described the construction and operation of a pilot plant in which whole wheat mashes were successfully fermented by *Aerobacillus polymyxa*. A yield of 8.9 lb. of *levo*-2,3-butanediol and 5.9 lb. of ethanol per bushel of wheat was obtained, which is a fermentation efficiency of 90%. The best fermentations were obtained at 32°C. using a 15% mash buffered with calcium carbonate. Agitation increased the fermentation efficiency at 96 hr. but decreased the diol-to-ethanol ratio. Reduced pressure did not change the efficiency but markedly reduced the ratio of diol to ethanol. Aeration had little effect. The presence of contamination organisms was highly detrimental and it was necessary to sterilize the mash, vessels, and piping. Although a backstocking technique could be practised in the preparation of inoculum, frequent recourse to stock cultures or improved strains was found advisable for maintenance of high yields.

Introduction

Laboratory studies on the production of 2,3-butanediol (subsequently referred to as diol) by the fermentation of wheat with *Aerobacillus polymyxa* were begun in these laboratories in 1942. Keen interest in the chemical and physical properties of this four carbon compound and its possible commercial use as a precursor for butadiene and as an antifreeze (5, 6, 8, 10) made it advisable to continue production and recovery studies beyond the laboratory stage. Wartime shortages and unavoidable delays prevented operation of the pilot plant until early in 1944. A small scale production unit was, however, in operation before this time (11). The data and experience obtained were applied to the larger pilot plant.

The object of the work was to determine the yields obtainable under optimum pilot plant fermentation conditions, and to produce material for recovery studies. The basic fermentation requirements had been investigated and reported previously (1, 2, 3, 4, 12). Whole wheat, when sterilized and buffered, had been found to be a satisfactory substrate, and laboratory methods of preparation were modified for pilot plant work. Considerable difficulty was experienced in obtaining noncontaminated fermentations but methods of sterilizing the mash, cooker, fermenters, and piping and of maintaining them

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sterile were developed. A vigorous strain from initial isolations was selected and throughout pilot plant work new selections from this and other strains were made. The results of inoculum studies in the laboratory were applied to pilot plant work and adjusted to routine conditions. When these basic requirements had been met the effects of various fermentation conditions were investigated. This paper deals only with the fermentation aspects of the pilot plant study while an earlier paper (13) is concerned with recovery studies.

Equipment

The equipment consisted of storage bins, grinding and weighing apparatus, slurrying equipment, a large and a small cooker, two progagators, two fermenters, and a beer well. A mash cooler was available but was not always used.

The storage bins were constructed of wood and sectioned to provide space for grain, malt, and calcium carbonate. The grain was ground in a small Greey attrition mill with an adjustable grind and a capacity of 12.5 bu. per hr. Screen analyses of the ground grain showed 4% coarser than 10 mesh, 62% between 10 and 20 mesh, 22% between 20 and 40 mesh, and 12% finer than 40 mesh with 2% passing 100 mesh. The weighing hopper of 1000 lb. capacity was made of galvanized sheet metal with sloping sides. Some of the ground grain stuck to the sides, but a standard vibrating mechanism would eliminate this objectionable feature.

A diagram of the cooking and fermenting equipment is given in Fig. 1. The slurrying tank was approximately 2 ft. in diameter and 2.5 ft. high with a capacity of 50 gal.* Agitation was provided by two 7 in. propellers spaced 8 in. apart driven at 383 r.p.m. A 4 in. screw conveyor rotating at 11.4 r.p.m. carried the grain from the weighing hopper to the slurrying tank at a rate of 510 lb. per hr. Water was metered manually to the tank; the rate was measured by a rotameter and total flow by a rotary piston meter. The resulting slurry was pumped from the tank to the tops of the cookers with an open impeller centrifugal pump.

The cookers were upright cylindrical vessels made of mild steel. Their conical bottoms were fitted with radial steam spargers with an additional sparger immediately above the bottom discharge valve. The large cooker had a capacity of 800 gal. It was equipped with a vertical rake agitator driven at 12 r.p.m. with the rakes spaced at 12 in. intervals in a spiral arrangement. The slurry feed pipe extended some distance into the cooker to prevent the mash from splashing on the walls of the vessel above the liquid level. The small cooker had a capacity of 50 gal. and was fitted with a nozzle mounted propeller mixer, driven at 1725 r.p.m. The cooked mash was blown by steam pressure through a pipe which extended from the bottom of the cookers, through the top, and then to the fermenters and propagators.

* *Imperial gallons are used throughout.*

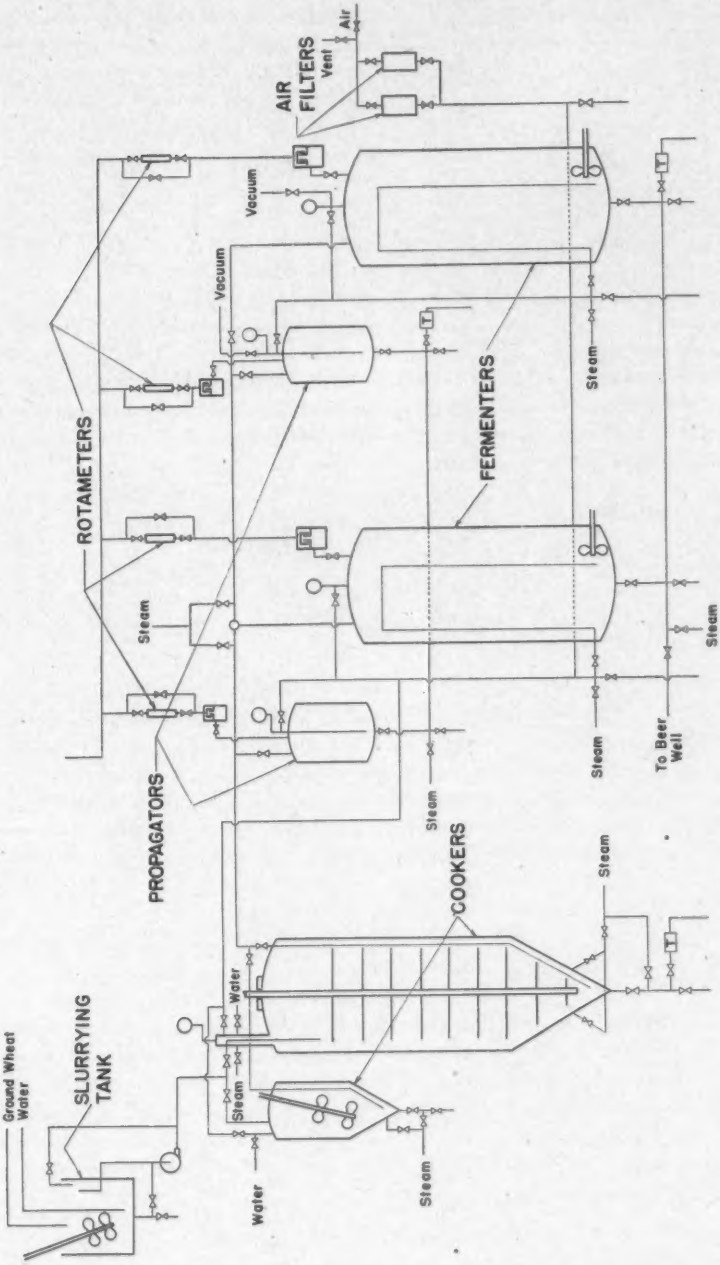

FIG. 1. *Pilot plant mashing and fermenting equipment.*

The two fermenters, identical in design, were of the deep vat, closed type. Their total capacity was about 750 gal., and a mash volume of about 450 gal. was used. Mash entered at the top and could be drawn off through a 2 in. discharge line at the bottom. Inoculum was added through a short 3/8 in. pipe at the top of the fermenter and samples were removed through a 3/8 in. line extending into the mash and curved downward above the fermenter. These are not shown in Fig. 1. A side entering agitator, located 1 ft. from the bottom of the cylindrical section, drove a three bladed, 12 in. diameter propeller located 1 ft. inside the tank. A seal cup beside the packing gland was filled with formalin to aid in preventing contamination. Sterile air entered the fermenters near the bottom, and various spargers could be connected. The final system adopted was to attach porous alumina aerators to the end of the air line shown in Fig. 1. These aerators had to be removed and cleaned after each fermentation in order to prevent them from plugging, and with the arrangement shown it was possible to disconnect the pipe and remove the aerators through the manhole. For gas discharge each fermenter had a separate water seal and scrubber.

The temperature was controlled by spraying thermostatically controlled water on the outside walls of the lower parts of the fermenters. The water was collected and recirculated. The fermenters had a minimum number of openings, and all connected pipes were lagged and kept under steam pressure when not in actual use. The tops of the fermenters were insulated to prevent heat loss and condensation.

The propagators were similar in design to the fermenters and each had a total capacity of 50 gal. They were supplied with steam, air, and temperature control using internal copper coils, but not with mechanical agitators. Each propagator had an individual water seal scrubber. These vessels were originally used to propagate inoculum for the fermenters but when it became evident that much smaller amounts of inoculum were as effective, the propagators were disconnected from the fermenters and used to test certain factors on a smaller scale.

The beer well was an open 1000 gal. tank equipped with a single paddle agitator. The fermented mash from both fermenters could be held in the beer well.

Auxiliary services to the plant provided water from the city mains, electrical power at 110, 220, and 550 v. and steam. A maximum steam pressure of 75 p.s.i. gauge was obtainable while the average was 50 p.s.i. gauge. Compressed air was available from either of two sources giving 50 and 100 p.s.i. gauge. Sterile air was obtained by passing compressed air through packed glass wool which was periodically steam sterilized. Reduced pressure was obtained with a small vacuum pump.

Experimental

Preparation of Slurry

The grain was elevated with a bucket elevator to the top floor and 650 lb. ground into the weighing hopper. The slurrying tank was filled with water and ground grain added from the hopper at a constant rate with the screw conveyor. Water was added to the tank at 4 gal. per min. and the liquid level in the tank was kept constant at the level of the overflow. The slurry that entered the overflow pipe was pumped to the top of the cooker. When all the grain had been added the slurrying tank was pumped out and rinsed with water. If calcium carbonate was to be added before sterilization it was mixed with the wheat in the hopper. If it was to be sterilized separately it was mixed with water in the slurrying tank and pumped to the small cooker.

Originally the ground grain was dumped directly from the hopper into 260 gal. of cold water, previously added to the cooker. The cover was replaced and cooking commenced. This system had a number of disadvantages. The grain dust spread rapidly and in addition to being a possible source of contamination this was very objectionable. In the cooker the fine particles settled on the walls above the water line, where they were cooked into a hard scale, providing another probable source of contamination. In addition, a poor mix was obtained as the grain clumped and tended to settle out, thus lowering sterilization efficiency. To overcome these difficulties the slurry tank was installed to provide rapid and thorough mixing.

Cooking and Sterilization

The most serious problem encountered was in obtaining consistently sterile cooks and uncontaminated fermentations. The thick, starch-gluten substrate complicated this problem. The most frequent cause of contamination was insufficient sterilization of the mash rather than the entrance of foreign organisms into the system after sterilization. The majority of the isolated contaminating organisms were spore formers. Only rarely were nonspore-forming contaminants encountered. James, Wilson, and Stark (7) report that whole wheat contains a natural flora that depends on the source, storage conditions, and grade. They found that the number of organisms was least with No. 1 Manitoba Northern, and progressively increased with successive lower grades with numbers ranging from 280,000 to 164,000,000 bacteria, 420 to 1870 fungi, and 6200 to 64,000 yeasts per gram. Over a period of time undesirable features and suspected sources of contamination were either removed or modified. The insertion of the slurry tank and changes in piping, which simplified connections but made the plant less versatile, solved the greater part of the problem.

To liquefy the thick mash, 2 lb. of ground malt was added. Initially, the malt was added to the slurry in the cooker and the temperature held at 70°C. for 20 min. before sterilizing. The final method adopted was to add the malt at the beginning of the slurrying operations, and, when these were complete,

to heat immediately to the sterilization temperature. Both methods gave a satisfactory reduction in viscosity but the latter was much simpler.

To heat the mash, steam was forced through the spargers at the bottom of the cooker. The temperature was followed on a recording thermometer. When all the air had been expelled from the cooker the vent valve was closed and the mash heated to the sterilization temperature. In test operations, 25 lb. of calcium carbonate was added to the mash and the whole heated to temperatures of 100°, 115°, 120°, and 125°C. for ½, 1, and 1½ hr. The lower temperatures and shorter periods produced nonsterile mashes while higher temperatures and longer times produced caramelized mashes which fermented slowly or not at all. Cooking the mash at 115°C. for one hour appeared to be most suitable but even so four out of ten successive cooks were not sterile. Caramelized compounds are known to be more readily formed at alkaline pH levels. The pH of the mash sterilized with calcium carbonate was found to be 7.0 or slightly higher, while that of the grain slurry alone was approximately 6.0. By sterilizing the calcium carbonate separately the grain could be heated at 120°C. for one hour without observable caramelization. This treatment produced consistently sterile mashes and with a few exceptions subsequent contaminations were traceable to other sources. The lower grades of wheat were utilized for the fermentation, and it was thought that if exposing the grain to ultraviolet light would reduce the numbers, especially the spore load, sterile mashes might be obtainable with shorter cooking periods. Whole wheat was passed over a polished vibrating plate 4 ft. long at the rate of 10 lb. per min. and subjected to radiation from four 18 in. G.E. germicidal lamps. A reduction in the spore count from 3500 to 250 per gram was obtained. The grain, however, still had to be ground, weighed, and slurried before it entered the cooker, having ample opportunity to become reinfected. After grinding and weighing operations the spore count had increased to 1000 per gram. Although irradiating the ground grain immediately before slurrying was recognized as advantageous, it was not considered practical because of the flour and grain dust.

With irradiated grain a reduction of 15 min. in cooking time was possible but a further reduction resulted in a contaminated cook. No detrimental effect on the fermentation appeared to result from the action of ultraviolet light on the grain with the three treated cooks tested.

Prior to slurry operations a fermenter was closed and sterilized with steam at 20 to 30 p.s.i. gauge. Steam was bled from all openings to ensure that air and condensate were removed. One and a half to two hours was sufficient time for sterilization. When the mash had been sterilized for the proper time it was blown over with steam pressure to the fermenters. The fermenter was cooled with cold water during the transfer and its pressure kept low. A sudden rise in fermenter pressure indicated that all the mash had come over from the cooker. The mash lines were immediately flushed with steam and again put under pressure. Steam pressure was maintained in the connecting line at the bottom of the fermenters at all times except when it was used to transfer mash.

While the mash cooled, sterile air was bled into the fermenter to maintain a positive pressure.

When the mash reached a temperature of 32°C., 25 lb. of calcium carbonate, previously slurried with 25 gal. of water and sterilized in the small cooker at 120°C. for one hour, was transferred to the fermenter in much the same manner as the mash. The fermenter temperature was then automatically controlled at 32°C. A low positive pressure was maintained until just prior to inoculation when the pressure was reduced to atmospheric and the inoculum added aseptically. As a check on operational technique the mash was allowed to incubate for 12 to 18 hr. before inoculation. Any contaminants then had an opportunity to multiply and could be easily detected. After inoculation, the fermentation proceeded at atmospheric pressure under controlled conditions. When the fermentation was complete, it was discharged into the beer well where it was held pending recovery operations.

Selection of Strain and Preparation of Inoculum

The cultures that were previously isolated and found to be good producers under laboratory conditions (12) were used in the pilot plant. Variations between the strains were marked and it was found advisable to reselect and test periodically. Selections from strain C42 (3) were used with good results in the pilot plant over a long period of time.

The cultures were stored at 3°C. on wheat agar slants, on slants under mineral oil, in sterilized soil, and at room temperature after lyophilization. All the methods appear to be equally satisfactory, but the mineral oil technique, because of its good preservation properties and simplicity, was finally adopted as routine. With this method an active culture was always available.

Heat shocking of sporulated cultures was tried but the results obtained with this treatment were somewhat erratic and the general trend appeared to be toward slightly lower yields. An attempt to acclimatize the organisms to diol and thus improve the yield was not successful. Five strains were incubated for 96 hr. periods in 15% wheat mashes containing 2% diol and in mashes containing increasing amounts of diol of 1% per transfer. After a series of 10 transfers a comparison with the original cultures of a normal 15% wheat mash was made. With the treated cultures the diol–ethanol ratio was raised from 1.3 to 2.3, but the combined yield was reduced from 4.2 to 3.6% products.

The original inoculation scheme involved three serial transfers from slant to propagator adding 6 liters of inoculum to 30 gal. of propagator mash. The mash was prepared in a manner similar to that described for the fermentation. After a 24 hr. incubation period this was transferred to the fermenter. The scheme gave an active inoculum which represented about 7% of the mash.

Laboratory tests (4) demonstrated that the amount of inoculum could be varied from 0.01 to 10% without affecting the yield and time required to complete the fermentation, although it did control the initial rate. In the pilot plant, adding inoculum at levels of 0.2, 0.1, and 0.05% gave completely

satisfactory results. The use of this low amount of inoculum is of importance in plant design as it eliminates large propagator equipment. On the above basis only 10 to 20 gal. of inoculum would be required for a 20,000 gal. fermenter. The practice adopted in the pilot plant was to add 2 liters of inoculum to the fermenter from a 4 liter aspirator bottle with an attached rubber hose and a metal guard tip which fitted into a 3/8 in. pipe on the fermenter.

The inoculum was prepared from agar slants by three 24 hr. stages. Transfers were made to 10 ml. of medium, to 200 ml., and finally to the 2000 ml. used to inoculate the fermenter. A backstocking technique was practised in the 200 ml. stage. Although backstocking through a series of six transfers had previously been found to give consistent yields (4), it was observed in the pilot plant that after a number of fermentations the yields and efficiencies decreased. In a supplementary laboratory study and under somewhat different conditions from the previous one, five strains were backstocked through 11 serial transfers and the diol and alcohol yields recorded after each transfer. A 15% mash distributed in 300 ml. amounts in 500 ml. Erlenmeyers was used with an incubation period of 96 hr. between transfers. On the eighth and ninth transfer the yields were observed to decline (Table I). Thus, in pilot plant operations, recourse was frequently made to stock cultures or to improved strains obtained by selecting and testing colonies from streak plates of fermenter samples.

TABLE I

THE EFFECT OF BACKSTOCKING IN 15% GRAIN MASHES

Backstock	Yield, %		
	Diol	Ethanol	Total products
1	2.22	1.40	3.62
2	2.30	1.43	3.73
3	2.16	1.37	3.53
4	2.40	1.47	3.87
5	2.50	1.33	3.83
6	2.20	1.36	3.56
7	2.24	1.28	3.52
8	1.99	1.08	3.07
9	2.04	1.09	3.13
10	1.94	1.06	3.10
11	1.92	1.10	3.02

The inoculating medium consisted of 1% ground wheat, 3% starch, 1.5% yeast extract, and 1% calcium carbonate. On this medium 500 million organisms per milliliter as estimated by direct count with a Petroff–Hauser plate were obtained after an incubation period of 24 hr. Malt sprouts or shorts offer a good commercial substitute for yeast extract as the added nutrient. Where no nutrient is added, growth is slower and numbers are much lower at 24 hr. The calcium carbonate may be omitted if the incubation period is not longer than 24 hr.

Fermentation

The effect of agitation, aeration, and reduced pressure on the yield and rate of fermentation was studied in the pilot plant. These factors had previously been studied in the laboratory (1, 3). It was shown that any treatment that assisted the escape of carbon dioxide increased the initial rate of fermentation with only a slight effect on the degree of completion at 96 hr. Thus a high surface–volume ratio, agitation, reduced pressure, and aeration with air, oxygen, nitrogen, or hydrogen increased the initial rate, while conducting the fermentation in an atmosphere of carbon dioxide decreased the initial rate. Aerobic conditions, such as a high surface–volume ratio, and aeration with air or oxygen increased the diol–ethanol ratio, while anaerobic conditions, such as reduced pressure and aeration with nitrogen, hydrogen, or carbon dioxide, decreased the ratio. The pH level of the fermentation in the range 5.8 to 7.0 was found (2) to have little effect on the yield or product ratio.

During the fermentation, samples were taken at 24-hr. intervals. Ethanol and diol analyses and a microscopic examination were made and the pH determined. The fermentation efficiency, ratio of diol to ethanol, yield of diol and ethanol in pounds per bushel, and relative value of the liquid products were calculated for noncontaminated fermentations from the 96 hr. analyses. The total weights of diol and ethanol in the fermented mash were calculated from the formulas given by Leslie and Castagne (9). The theoretical yield of diol plus ethanol is considered as one-half of the weight of glucose formed from the starch, and the fermentation efficiency is calculated as the actual yield of diol plus ethanol divided by the theoretical yield. The cost estimates described in a previous paper (13) established the cost of diol at about three times the selling price of ethanol. The relative value of the fermentation products is therefore calculated by multiplying the diol yield in pounds per bushel by three and adding the yield of ethanol. The average values obtained in a series of fermentations showed that the fermentations were 60 to 70% complete at 48 hr., 80% at 72 hr., and 90% at 96 hr. The pH was found to drop from 6.5 to 5.7 in the first 24 hr. with a final pH of 5.3 to 5.5.

The effect of agitation on the fermentation is shown by the results given in Table II. The effect of increasing the time of agitation is to increase the efficiency and to decrease the ratio of diol to ethanol. Twenty-four hours' agitation increases the efficiency appreciably but has only a small effect on the ratio. Agitation for 48 hr. reduces the ratio but does not increase the efficiency. The results for 72 and 96 hr. agitation showed only small differences so they have been combined. Continuous agitation for 96 hr. gives the products with the highest relative value but the difference is small.

The effect of reduced pressure is shown by the data in Table III. Most of the data are based on a single fermentation and hence definite conclusions cannot be drawn. The first portion of Table III gives the effect of increasing time of vacuum on fermentations agitated for 96 hr. The efficiencies vary considerably but the ratio and relative value tend to decrease. The second

TABLE II

THE EFFECT OF AGITATION ON THE FERMENTATION

Time of agitation, hr.	No. of runs	Efficiency,* %	Lb. per bushel		Ratio	Value*
			Diol	Ethanol		
0	19	85.0	8.50	5.52	1.54	31.0
24	8	88.2	8.73	5.83	1.49	32.0
48	5	87.3	8.38	6.01	1.39	31.2
72-96	11	92.8	8.65	6.65	1.30	32.6

* *See text for method of calculation.*

TABLE III

THE EFFECT OF REDUCED PRESSURE (15 IN. OF MERCURY VACUUM) ON THE FERMENTATION

Length of treatment, hr.	Time of agitation, hr.	No. of runs	Efficiency,* %	Lb. per bushel		Ratio	Value*
				Diol	Ethanol		
0	96	11	92.8	8.65	6.65	1.30	32.6
12	96	1	84.5	7.98	5.95	1.34	31.9
24	96	1	75.2	6.96	5.44	1.28	26.5
48	96	1	87.7	7.08	7.37	0.96	28.8
0	24	8	88.2	8.73	5.83	1.49	32.0
24	24	1	91.7	9.07	6.05	1.50	33.2

* *See text for method of calculation.*

TABLE IV

THE EFFECT OF AERATION ON THE FERMENTATION

Aeration		Agitation time, hr.	No. of runs	Efficiency,* %	Lb. per bu.		Ratio	Value*
Rate, c.f.m. per 1000 gal.	Time, hr.				Diol	Ethanol		
0	0	72-96	11	92.8	8.65	6.65	1.30	32.6
3.5	72-96	72-96	4	93.4	9.57	5.83	1.64	34.5
7.0	96	96	1	80.1	10.71	2.50	4.28	35.3
0	0	24	8	88.2	8.73	5.83	1.49	32.0
4.0	24	24	2	84.9	8.10	5.90	1.38	30.3
0	0	48	5	87.3	8.38	6.01	1.39	31.2
4.0	48	48	1	88.4	8.52	6.05	1.41	31.2

* *See text for method of calculation.*

portion of the table gives the effect of 24 hr. vacuum applied to fermentations agitated for 24 hr. The efficiency is increased slightly with only a small effect on the ratio.

Aeration data obtained with porous alumina aerators are given in Table IV. All fermentations were agitated throughout the aeration period. Aeration for 72 to 96 hr. increases the efficiency and ratio, giving a higher relative value. Aeration at a rate of 7.0 c.f.m. per 1000 gal. for 96 hr. caused a loss of ethanol, so that the efficiency is low and the ratio high. In spite of the loss of ethanol the relative value of the products is very high. Aeration for 24 or 48 hr. has only a slight effect on the efficiency and ratio.

Discussion

From the results given in Tables II – IV it is not possible to specify the best conditions for fermentations in much larger fermenters. The aeration results are not conclusive because of the small number of fermentations. However, the effect of aeration is less marked in the pilot plant than on a laboratory scale, so it may be concluded that the effect would be even less in large fermenters. Thus, it is not likely that increased yield of diol or saving of fermentation capacity would pay for the increased power requirements and the high initial cost of installing air compressors and aeration equipment. This also applies to the application of reduced pressure.

The installation of agitators would be advisable to facilitate removing the mash and for maintaining a constant and uniform temperature. If the cooked mash were to be cooled in the fermenters, agitation would be necessary. Thus an increased yield resulting from agitation would have to pay for only a portion of the power consumption in order to be justified. By installing an agitator with a low circulation rate and little shear the initial cost could be kept down. This might give the same effect and same power cost over a 72 or 96 hr. period as a larger agitator over a 24 hr. period.

Based on the results of Table II, a probable efficiency of 90% and a diol-to-ethanol ratio of 1.5 were used for subsequent cost estimates.

Acknowledgments

The authors wish to express their appreciation for the generous support and encouragement given this project by Dr. W. H. Cook, Director of the Division of Applied Biology. Of those who contributed to the design and operation of the pilot plant, Dr. G. A. Adams, D. Rose, W. S. King, and I. M. Miller deserve special thanks. Acknowledgment is also made for the co-operation and invaluable assistance given by the technical and maintenance staff.

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THE OXIDATION, IGNITION, AND DETONATION OF FUEL VAPORS AND GASES

VIII. THE CAUSES OF THE ANTIKNOCK PROPERTY OF RICH MIXTURES[1]

By R. O. King,[2] W. A. Wallace,[3] E. J. Durand[4]

Abstract

The engine experiments of this Part are a sequel to those of Part VII showing that enrichment of a pentane–air mixture accelerates oxidation at end gas temperatures to the antiknock substances, steam, and the carbon oxides. The corresponding antiknock effect in an engine is increased by cooling if enrichment of the mixture leads to an increase in the proportion of the fuel admitted to the engine as liquid. The engine experiments were therefore made using two fuels, *n*-pentane and a commercial fuel "S", containing high boiling point constituents. The pentane could be vaporized prior to admission to the engine and the antiknock effect due to cooling eliminated. Thus, two concurrent antiknock effects were obtained on enriching the fuel–air mixture—one due to cooling, if the fuel were admitted to the engine in part as liquid, and the other to the consequent increase in the velocity of the heterogeneous oxidation reaction in the end gas. It was also shown by the experiments of Part VII that the oxidation of rich mixtures at end gas temperatures, to steam and carbon dioxide, was greatly accelerated when iron carbonyl was added to the fuel. Similarly, the engine experiments of this Part show that the antiknock effect of enriching the fuel–air mixture is greatly enhanced when iron carbonyl is added to the fuel.

Introduction

It has long been known that the tendency of a particular fuel–air mixture to detonate in an engine reaches a maximum for a critical mixture strength and that both weaker and richer mixtures possess antiknock properties.

When using ordinary gasolines, maximum power is obtained when the mixture with air is somewhat on the rich side of the critical mixture, and a further substantial increase of mixture strength permits the use of relatively high compression ratios without detonation, though at some sacrifice of economy. Alternatively, compression ratio being fixed, as in practice, the use of rich mixtures makes possible an increase of charge density by supercharging without giving rise to the detonation which would otherwise limit the consequent power increase. Thus the fuel for supercharged aero engines is now required to give a specified increase in antiknock value, designated as "mixture response", for a particular increase in mixture strength.

The antiknock property of rich mixtures is inconsistent with currently accepted views that detonation or knocking combustion is due to an oxidation

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reaction of the chain type, beginning with the formation of organic peroxides and proceeding to explosion in a homogeneous mixture, and that metallic antiknocks act by breaking the reaction chains. Organic peroxides are formed most readily in rich mixtures (2, p. 211) and if the antiknocks acted by destroying them or in any other way breaking the chains, the efficacy of a particular concentration of antiknock would diminish with increase of mixture strength. The opposite effect is observed in practice.

The antiknock property of rich mixtures has accordingly been given scant attention in attempts to apply chain reaction theory to oxidation as it occurs in an engine and the few available references are to the cooling effect. Thus Callendar (3, p. 511) explains that overrich mixtures reduce detonation by retarding ignition and lowering the engine temperature. Egerton (5, p. 2911) after mentioning the effect of enrichment of the mixture to increase rate of oxidation, which on the basis of his chain reaction theory would *promote* detonation, attempts to dispose of the anomalous antiknock property by suggesting that the proknock effect due to oxidation is offset by an antiknock effect due to cooling. Campbell, Lovell, and Boyd (4) describe experiments showing the importance of mixture strength in respect of the rating of fuels for antiknock value but do not attempt an explanation of the observed effects. Finally, Beatty and Edgar (1) in a lengthy review of "The theory of knock in internal combustion engines", describing in detail the factors influencing the promotion or prevention of the effect, make no mention of the antiknock effect due to enrichment of the mixture or the increase in the efficacy of the metallic antiknocks when used in rich mixtures; both effects are in contradiction to the chain reaction theories advanced.

Plan and Scope of the Experiments

Ordinary liquid fuels are more or less "atomized" in the carburetor of an Otto cycle engine. Vaporization requires the addition of heat and occurs mainly in the heated induction system and in the hot cylinder, where it is assisted by the hot residual gas. Any antiknock effect due to cooling by the fuel–air mixture is caused solely by the vaporization of the fraction of the fuel entering the cylinder as liquid. Experiments were planned accordingly, using two fuels, technical grade *n*-pentane and fuel "S". The initial boiling point of the pentane was 95°F.; 90% was recovered at 96.4°F. and 95% at 97.4°F., the end point. The initial boiling point of fuel S was 85°F. and 97% was recovered at 429°F., the end point (see Fig. 1). Distillations were in accordance with A.S.T.M. procedure, D86—46.

The pentane even in rich mixtures with air could be vaporized prior to admission to the cylinder and any antiknock effect then observed on enrichment of the mixture could not be due to cooling. Fuel S could be admitted almost entirely as liquid or could be vaporized in part only with the means available, prior to admission to the cylinder. Thus by using the two fuels and varying the rate of heat addition to the mixtures with air, conditions could be

obtained in which nearly all of the fuel could be admitted to the engine as vapor or nearly all as liquid, and the consequent effects on detonation determined by varying the compression ratio to maintain a standard knock intensity.

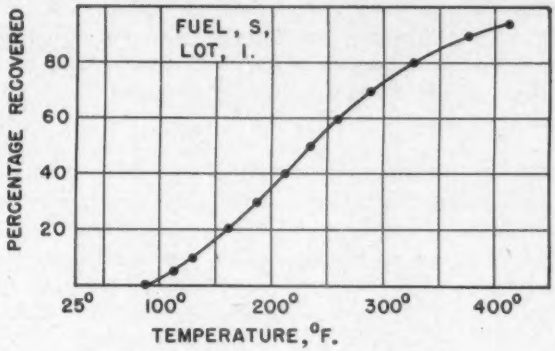

FIG. 1. *Distillation range, Fuel S, Lot 1.*

The scope of the experiments was extended to determine the antiknock effect of enrichment of mixtures with air of fuel S containing iron carbonyl in concentrations extending to 1.0 cc. per liter.

Experimental Arrangements

A standard C.F.R. unsupercharged variable compression engine was used for the experiments. The bore and stroke are 3.25 in. and 4.50 in. respectively and the compression ratio can be varied from 4 to 10 : 1. The engine is in universal use and further details need not be given although certain special arrangements require description.

Engine Cooling

The standard evaporative arrangement for maintaining the jacket water at a constant temperature of 212°F. was on occasion replaced by a cold tap water circulation, the flow being regulated manually to maintain any desired lower temperature.

Mixture Strength Regulation

The float chamber is flexibly connected to the carburetor, and mixture strength is varied by varying the height. When the chamber is in the "up" position, the surface of the fuel in it is level with the fuel opening into the carburetor throat, and maximum mixture strength is obtained. When the chamber is in the "down" position, the fuel level is 3.0 in. lower and mixture strength is generally too weak for engine operation. The rate of fuel consumption at a particular engine speed then depends on float chamber level, the density and velocity of the air passing through the carburetor throat, the vapor pressure of the fuel, and the diameter of a flow control orifice fitted in the fuel line from the float chamber to the carburetor.

Measurement of Mixture Strength

The C.F.R. knock testing engine is not provided with a fuel flow meter. The mixture strength required for maximum knock having a critical value for any particular fuel, the percentage variation from it can be calculated if corresponding rates of fuel consumption be measured. Fuel flow metering arrangements made accordingly were based on observations of the time required for the consumption of a particular weight of fuel, and were similar to those of the Waukesha Motor Co. (8).

Mixture Temperature and Fuel Vaporization

The air supply to the carburetor was at room temperature and humidity was not controlled. The mixture of air and liquid fuel passed from the carburetor through a heating chamber to the inlet passageway in the engine head. A requirement of the C.F.R. "motor method" of knock testing is that the combustible mixture be raised to a temperature of 300°F. prior to admission to the engine, and it is necessary in order to avoid oxidation and possible pre-ignition that the mixture be brought into contact with moderately heated surfaces of relatively large area. The effective length of the heating chamber is 7.0 in. and the inside diameter 1.72 in. Two electric heating elements each 6.75 in. long, 1.06 in. wide and 0.36 in. thick are fitted in the chamber. The area of electrically heated surface is 39.5 sq. in. and that of the surrounding surface heated by radiation is 44 sq. in. There is, in addition, about 12 sq. in. of heated induction passageway surface in the cylinder head leading to the inlet valve.

It is a requirement of the scheme of the experiments that *n*-pentane be completely vaporized prior to admission to the cylinder when the mixture temperature is 300°F. and the jacket temperature 212°F. The heat input in these conditions was 47 B.t.u. per min. and of this 6 were required to vaporize the pentane at the maximum rate of consumption and 26 to heat the air, leaving a balance of 15 for radiation and conduction. It is a fair assumption, therefore, that the mixture heating arrangements suffice to vaporize completely the *n*-pentane in a mixture with air raised to 300°F. even in the time available, when it is considered that the volume of the heater space was approximately 50% of that of the stroke volume of the engine and that the vapor pressure of the pentane at the laboratory temperature at the time of the experiments was approximately 600 mm. of mercury.

Mixture temperatures given later and on relevant graphs were as indicated by a mercury-in-glass thermometer with the bulb in a pocket in the short passageway from the heating chamber to the inlet port of the engine.

Ignition Timing

The standard C.F.R. method of changing ignition timing with change of compression ratio was used and timing was varied from 33.5° advance at 4:1 compression ratio to 14.7° advance at 10:1, but the relation is not linear as shown by the graph of Fig. 2.

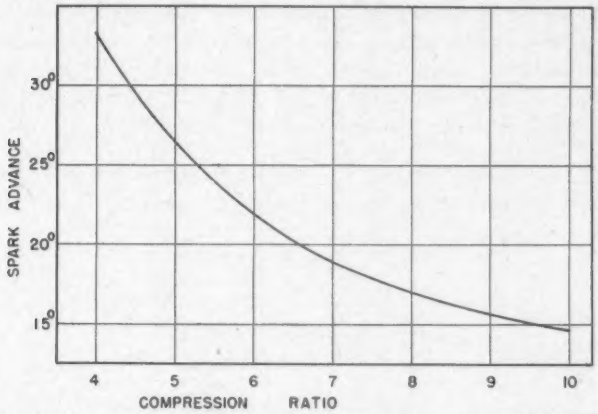

FIG. 2. *Variation of spark advance with compression ratio, C.F.R. engine.*

Engine Speed

The C.F.R. engine is usually arranged to drive a synchronous generator connected to an a-c. supply of constant frequency. The engine speed is thus maintained constant regardless of power output. The C.F.R. engine used for the experiments was connected by a belt drive to a d-c. generator and electrical output absorbed by a resistor bank. The engine speed was maintained at *900 r.p.m. for all of the experiments* by manual regulation of the field resistance.

Standard Knock Intensity

The C.F.R. bouncing pin device and knock meter were used for determinations of an arbitrarily chosen "standard knock intensity" equivalent to that obtained in C.F.R. knock testing practice when using a standard 75 octane fuel at a compression ratio of 5.26. The calibration of the bouncing pin was always checked accordingly before starting experiments.

Inlet Valve

A shrouded inlet valve is fitted as standard to the C.F.R. engine. The valve imparts a swirl to the entering mixture but the consequent restriction reduces volumetric efficiency. It was replaced by a spare exhaust valve of the common tulip shaped variety.

Lubrication

The engine was lubricated with a commercial brand of oil, S.A.E. 30, without "additives". The oil in the crank case was maintained at temperatures between 120° and 130°F., by a manually controlled electric heater.

Experimental Results

Experiments were made with a jacket water temperature of 212°F. obtained by the evaporative cooling method already mentioned and with a jacket water temperature of 150°F. obtained by the circulation of tap water, as indicated

by a thermometer with the bulb in a pocket in the circulating water *outlet* of the cylinder head. The term "jacket temperature" is used for convenience to describe temperature conditions as above. It will be understood that the corresponding cylinder wall and combustion space surfaces are higher to an unknown extent, depending on variable heat transfer factors.

The rate of fuel consumption was varied from the minimum at which the engine would run steadily to the maximum obtainable with the C.F.R. 0.027

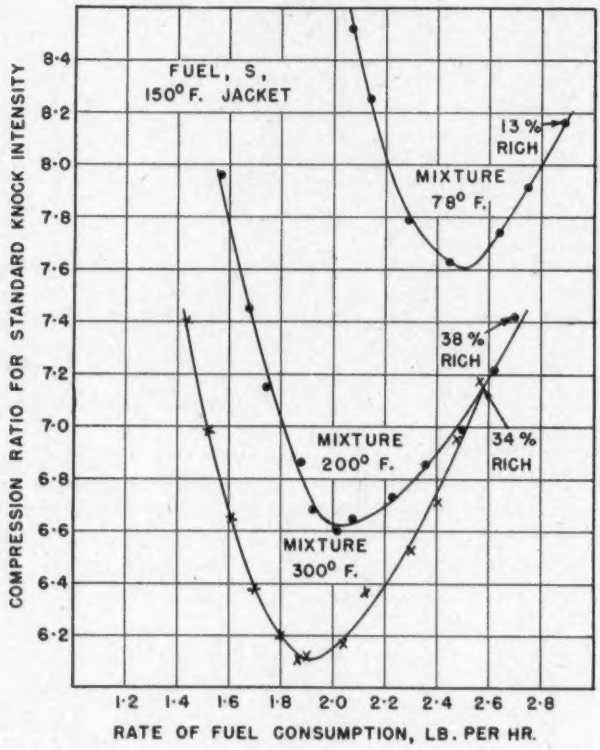

FIG. 3. *Fuel S, Lot 1. 150° F. jacket temperature.*

in. dia. fuel control orifice usually fitted for use with hydrocarbon fuels. Observations were thus obtained of the antiknock effects due to weakening as well as to enriching the mixture. The use of weak mixtures was of beneficial effect in burning the carbon deposited during previous running of very rich mixtures. Thus when the experiments were completed, the combustion space was found to be remarkably clean and both valves to have been seating properly. The piston rings were in good condition and free in the grooves. There was little carbon in any of the grooves. The piston crown was coated

with an adherent layer of carbon; 0.002 in. thick at the center, shading to black at the periphery and increasing in thickness to about 0.005 in.

The coating on the inlet valve was similar to that on the piston crown. There was a reddish coating about 0.005 in. thick on the exhaust valve and a similar coating on the water cooled surfaces. The color is attributed to iron oxide derived from additions of iron carbonyl to fuel S.

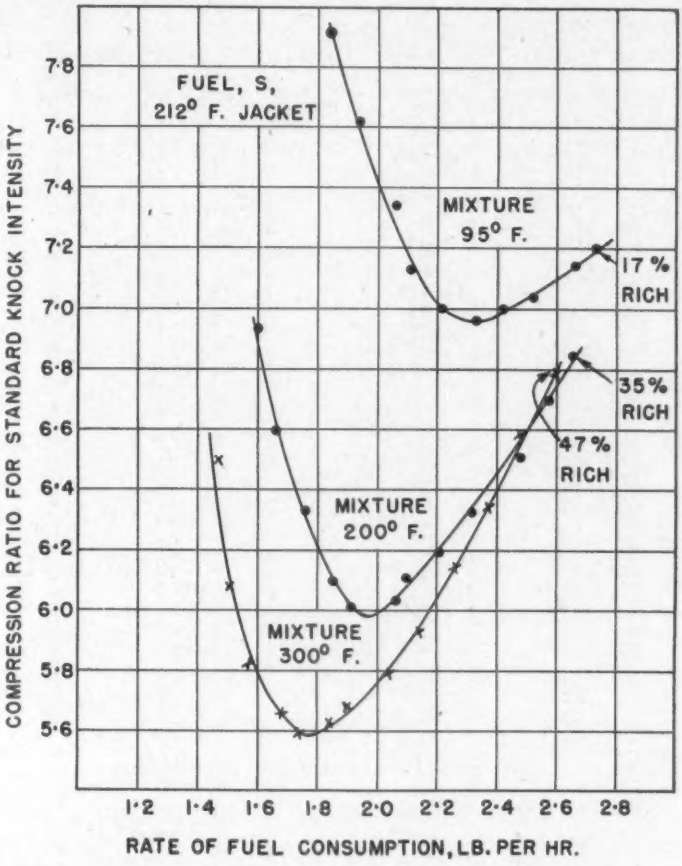

FIG. 4. *Fuel S, Lot 1. 212° F. jacket temperature.*

Standard intensity of knock was determined for rates of fuel consumption varying by steps over the range possible in the flow control conditions already described, with the mixture unheated, and at temperatures of 200° and 300°F. and with jacket temperatures of 150° and 212°F. The experimental results are given by the graphs of Figs. 3, 4, 5, and 6. It will be noted that the rate

of fuel consumption required for the critical mixture strength decreases with increase in mixture temperature as would be expected in view of the consequent decrease in the *weight* of air aspirated per stroke.

Experiments with Fuel S

The fuel contains volatile fractions in some proportion to provide for ease of motor car engine starting, but the graph of Fig. 1 shows that 35% only boils at temperatures below 200°F. and 74% at temperatures below 300°F. The corresponding vapor pressures and the depression in the carburetor throat determine the maximum rate of fuel consumption when the float chamber is in

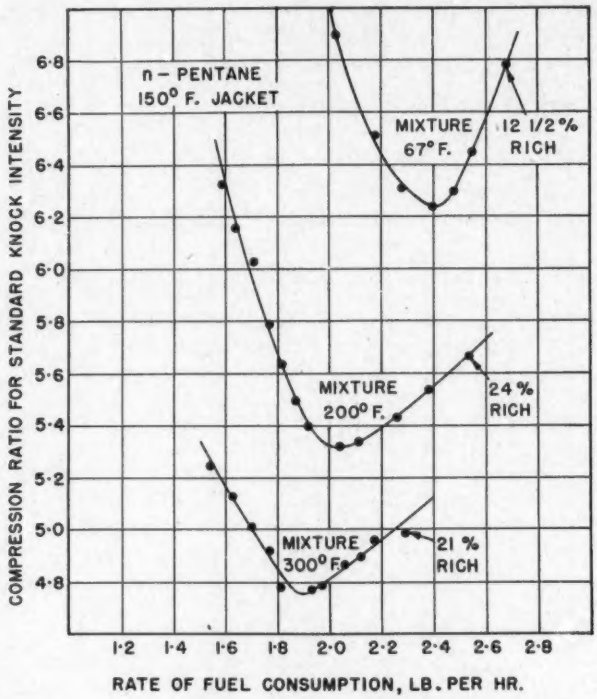

FIG. 5. *n-Pentane. 150° F. jacket temperature.*

the "up" position. The maximum rate possible with a particular diameter of flow control orifice therefore diminishes with increasing mixture temperature. Thus, referring to experiments at 150°F. jacket temperature, Fig. 3, the maximum rate of fuel consumption diminished from 2.89 to 2.56 lb. per hour as mixture temperature was increased from 78° to 300°F. In the same circumstances the rate of fuel consumption required to maintain the critical mixture strength, at which the minimum compression ratio giving standard knock intensity occurs, diminished from 2.50 to 1.90 lb. per hr. The maximum

mixture strengths obtainable varied accordingly as shown by the percentages given on the graphs.

Experiments with n-Pentane (Figs. 5 and 6)

Pentane was selected as the second fuel because of the expectation that the mixture heating means available would suffice for complete vaporization prior to admission to the engine cylinder. The experiments were made during hot summer weather and there was generally a tendency to form vapor in the fuel

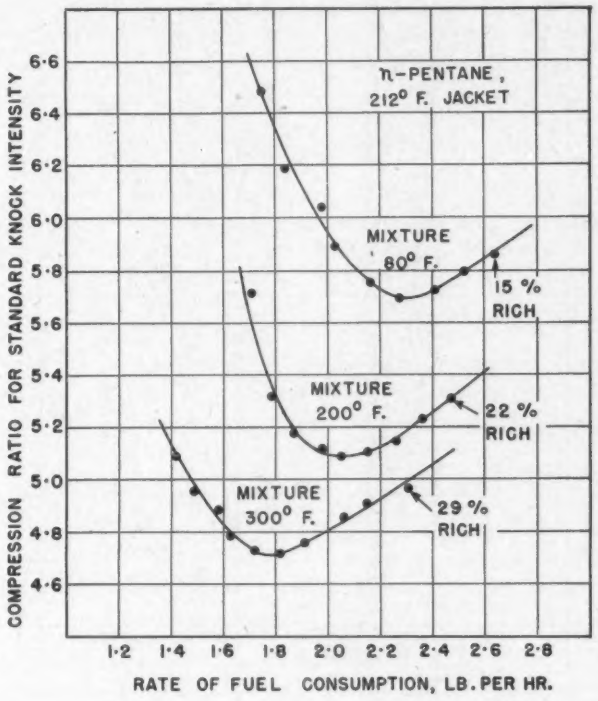

FIG. 6. *n-Pentane. 212° F. jacket temperature.*

line to the carburetor. On one occasion when the official Toronto shade temperature was 101°F., the pentane boiled in the float chamber and the experiment could not be continued.

The relatively high vapor pressure of the pentane assisted vaporization but placed a relatively low value on the maximum possible rate of consumption because of the lower than atmospheric pressure in the carburetor throat. Thus at 212°F. jacket temperature and 300°F. mixture temperature, Fig. 6, the maximum rate of pentane flow was 2.30 lb. per hr. whereas when using the less volatile fuel S the maximum flow was 2.60 lb. per hr. The degrees of

enrichment obtained in the conditions of the experiments are shown on the graphs of Figs. 5 and 6 and suffice for the purpose of the investigation.

Experiments with Fuel S Doped with Iron Carbonyl

The experiments were carried out with the cylinder jacket at 212°F. and the mixture at 300°F. The fuel was from a lot of 50 gal. of old stock and differed

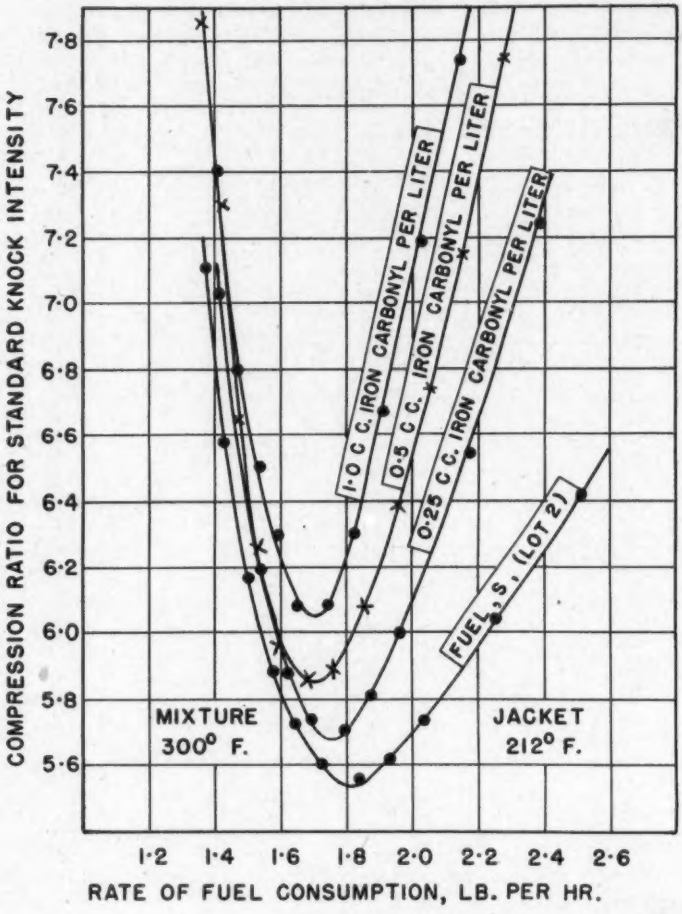

FIG. 7. *Fuel S, Lot 2, and with iron carbonyl in concentrations increasing to 1.0 cc. per liter.*

slightly in antiknock value and considerably in "mixture response" from that used previously which was obtained as required from a local service station.

The influence of enriching the mixture on the antiknock effect of iron carbonyl is shown by the graphs of Fig. 7. The increase in the antiknock

effect obtained on increasing mixture strength is quite remarkable. Thus the compression ratio for standard intensity of knock when using the undoped fuel at the critical mixture strength is 5.54, rate of fuel consumption being 1.8 lb. per hr. but on increasing the rate to 2.2 lb. per hr. and adding iron carbonyl in the concentration of 1.0 cc. per liter, that is, one-tenth of one per cent, the compression ratio for standard intensity of knock rose nearly 2.5 compression ratios, to 8.0:1.

Generally similar results were obtained when the fuel was doped with tetraethyl lead or nickel carbonyl. Those for iron carbonyl are presently given because the substance was used in experiments described in Part VII (7), showing that enriching the mixture increases the oxidation promoting effect of the antiknock.

Discussion of the Experimental Results

It was shown by oxidations in reaction chamber No. 10, described in Part VII (7), that over the temperature range 600° to 650°C. reaction velocity increased by 100% on enriching a pentane–air mixture from 25% weak to 100% rich. The reaction products at the high temperatures were steam and the oxides of carbon. Reaction velocity was greatly accelerated by iron carbonyl, the temperature of any particular rate of reaction being reduced by as much as 150°C., and the reaction products were steam and carbon dioxide only. The high temperature oxidation products are all antiknocks and if present in the end gas of an engine would reduce the tendency to detonation or knocking combustion as discussed in Part III (6).

The antiknock property of *rich mixtures* is indicated by the slope of the graphs for mixture strengths greater than the critical value, that is, by the ratio,

$$\frac{\text{Increase of compression ratio for standard knock intensity}}{\text{Increase in rate of fuel consumption}}.$$

The ratio will be designated by the letter, R. The experimental results summarized accordingly are given in Tables I, II, and III.

Pentane Boiling Range 95° to 97.4°F. (Table I)

The jacket temperature being 150° and the mixture temperature 67°F., $R = 2.4$; then on heating the mixture to 200°F., more of it is vaporized prior to entering the cylinder; the cooling effect diminishes accordingly and R decreases to 0.86. A further increase of mixture temperature to 300°F. reduces R to 0.70, indicating that the cooling effect has been reduced but not eliminated.

The results for a jacket temperature of 212°F. can now be considered. Starting with a mixture temperature of 80°F., the value of R is 0.70 only. It diminishes to 0.56 when the mixture is heated to 200°F. and remains un-

changed, within the accuracy possible, on further heating the mixture to 300°F. The conclusion is that the pentane was vaporized completely when the mixture temperature was raised to 200°F. and that the value of R then obtained represents the antiknock effect arising from the increase in rate of oxidation due to increasing the mixture strength by 29%.

TABLE I

EXPERIMENTAL RESULTS—PENTANE

Jacket temp., °F.	Mixture temp., °F.	Ratio R	Jacket temp., °F.	Mixture temp., °F.	Ratio R
150	67	2.4	212	80	0.70
150	200	0.86	212	200	0.56
150	300	0.70	212	300	0.54

Fuel S, Lot 1, Boiling Range 85° to 430°F. (Table II)

The jacket temperature being 150°F. and the mixture temperature 78°F., vaporization would occur mainly in the cylinder, the maximum cooling effect would be obtained, and $R = 1.7$. The large increase of mixture temperature to 200°F. reduces R to 1.6 only and on further increasing mixture temperature to 300°F., R *increases* to 2.1. Thus, as the proportion of the fuel vaporized

TABLE II

EXPERIMENTAL RESULTS—FUEL S (LOT 1)

Jacket temp., °F.	Mixture temp., °F.	Ratio R	Jacket temp., °F.	Mixture temp., °F.	Ratio R
150	78	1.7	212	95	0.6
150	200	1.6	212	200	1.6
150	300	2.1	212	300	2.5

outside the cylinder increases and the cooling effect diminishes accordingly, R increases. This characteristic is more pronounced when the jacket temperature is raised to 212°F. and the initial mixture temperature is 95°F. R is then 0.6, increases to 1.6, and to 2.5 as mixture temperatures are raised first to 200°F. and then to 300°F. That is, the antiknock effect of enrichment *increases* as the evaporative cooling effect diminishes. This characteristic is attributed to the relatively great susceptibility to oxidation of the higher boiling point constituents of the fuel.

Fuel S, Lot 2, Plus Iron Carbonyl (Table III)

The experiments were made at jacket and mixture temperatures of 212° and 300°F. respectively. The high value of R due to the susceptibility to oxidation of the heavier fractions of the fuel is then increased by the oxidation promoting effect of the metallic antiknock. The value of R increases accordingly from

1.4 for the undoped fuel to 4.4 for a dope concentration of 0.50 cc. of iron carbonyl per liter. Little further increase of R is obtained on doubling the dope concentration. This would be expected from the experimental results described in Part VII showing that the oxidation promoting effect of iron carbonyl reaches a limiting value as concentration and mixture strength are increased.

TABLE III

EXPERIMENTAL RESULTS—FUEL S (LOT 2) PLUS IRON CARBONYL, JACKET TEMPERATURE 212°F., MIXTURE TEMPERATURE 300°F.

Fuel	Ratio R
Fuel S undoped	1.4
Fuel S plus 0.25 cc. I.C. per liter	3.0
Fuel S plus 0.50 cc. I.C. per liter	4.4
Fuel S plus 1.00 cc. I.C. per liter	4.5

Conclusions

The experimental results are not in accordance with the current theory that knock or detonation in an engine is the result of an oxidation reaction in the end gas proceeding by a chain mechanism (1, 5). On the contrary, they support the view that knocking combustion tends to be prevented by dilution of the end gas with the antiknock products of the oxidation reaction occurring at the high temperature of the end gas (6), and it is concluded accordingly that:

(1) Enrichment of the fuel–air mixture used in an engine leads to an antiknock effect *in addition* to that due to the increased cooling, because of the consequent increase in the rate of oxidation of the end gas.

(2) The additional antiknock effect increases with increase in the susceptibility of the fuel to oxidation.

(3) The antiknock effect of iron carbonyl increases with increase of mixture strength because of the corresponding increase in the oxidation *promoting* effect of the substance, at end gas temperatures.

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SOME FACTORS AFFECTING THE CYANOGENETIC CONTENT OF FLAX[1]

By G. R. Paterson[2] and E. Y. Spencer[3]

Abstract

Many factors, both inherent and environmental, affect the linamarin content of flax (its cyanogenetic principle). Redwing variety contains considerably more potential cyanide than does Royal. Flax, maintained at a high moisture level throughout the growth season, contains significantly less linamarin than that grown with access to less soil moisture. Frost, mechanical injury, and drought all affect the cyanogenetic content of flax adversely, the effect of the first being very great. Flax grown at the higher moisture level was more affected by these conditions than was flax grown at the lower moisture level. The recovery of cyanide from the glycoside by simultaneous enzymatic hydrolysis and aeration, and its estimation by the alkaline silver nitrate method, is an effective laboratory means of assessing the cyanogenetic content of flax. Although acetone, one of the decomposition products of linamarin, normally reacts with the alkaline picrate reagent, picrate under certain conditions may be adapted to the roughly quantitative estimation of cyanide by test paper in the field.

Introduction

Robinson (12), Auld (2), and Henry and Auld (8) have reviewed the history of cyanogenesis in a very comprehensive manner, one (8) with special reference to linseed. Although cyanogenesis, the occurrence of hydrocyanic acid in the plant kingdom, was discovered in 1803, it was not until 1883 that flax was found by Jorissen to be cyanogenetic. In 1891, Jorissen and Hairs isolated the cyanogenetic glycoside, linamarin, and described its enzymatic hydrolysis to hydrogen cyanide, D-glucose, and a volatile ketone giving the iodoform reaction. Dunstan and Henry showed the aglycone was acetone cyanhydrin in 1903. Linamarin is accompanied in flax (in different cells) by a specific β-glycosidase, linamarase, which hydrolyzes it to its components, hydrogen cyanide, D-glucose, and acetone. In flax, the quantity of linamarase present usually parallels the content of linamarin.

Cyanogenesis is inheritable (3), the cyanogenetic character being governed by the interaction of genes determining the presence or absence of the glycoside and the enzyme. However, the amount of glycoside present in a cyanogenetic plant is subject to variation by many other factors. Any factor which changes the normal season of growth, e.g., drought, injury (such as in a hail storm), frost, or insect attack, increases the amount of potential cyanide (4, 6, 9, 11). Many forage plants are cyanogenetic and so constitute a possible threat to the stock who feed upon them. The toxic dose for most animals is the quantity of cyanogenetic glycoside equal to about 2 mgm. of hydrogen cyanide per pound

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of body weight (13). Based on the average cyanogenetic content of flax (25 mgm. % of hydrogen cyanide), a portion of less than 2 lb. would prove fatal to an animal weighing 100 lb., assuming complete hydrolysis of the glycoside. Under abnormal conditions of growth (frost especially), flax may contain two to three times as much potential cyanide and so constitute a serious menace to stock. However, many substances have an inhibiting effect on the release of hydrocyanic acid from linseed cake (2, 8), among them glucose, salt, and the crude fiber of certain green fodders. It is recommended that these be fed along with linseed cake or other cyanogenetic plants. The use of the defatted residue, after hot-press oil extraction of flax, should be encouraged, since it contains little active enzyme owing to its destruction at the temperature employed (8).

Methods and Materials

A greenhouse experiment was designed to assess the effect of various climatic factors as well as varietal effect on the cyanogenetic content of flax (10). Two varieties of flax were chosen—Royal and Redwing. The wilting level and moisture capacity of a soil mixture of seven parts of top soil, two parts of sand, and one of manure (subsequently used as the growth medium in the experiment) were determined as 15.5% and 28% respectively. It was decided therefore to grow both varieties at soil moisture levels of 18% and 27%, termed hereafter "low moisture" and "high moisture" levels. Each variety at each moisture level was submitted to four conditions, control, frost, injury, and drought, making a total of 16 treatments. Each treatment consisted of six pots containing an average of nine plants each.

Moisture levels were maintained by weekly watering, distribution of the water evenly in the soil mixture being aided by means of a sand column. The conditions of frost, drought, and injury were attained or simulated in the following manner. A month after first flowering, the plants which were to be frosted were placed in a separate section of the greenhouse, where, over the course of four days, the temperature was gradually lowered to 32°F. On the evening of the fourth day, the temperature was allowed to fall still lower, reaching a minimum of 24.5°F. The plants were allowed to proceed towards maturity, having been submitted, after a hardening process, to freezing temperatures for four to five hours. In the drought experiments, 10 weeks after planting, the plants were withdrawn from their normal weekly watering schedules, which were not resumed until the plants had reached the wilting levels. The procedure was repeated before the plants were allowed to go on towards maturity with the regular moisture levels. A week to ten days after flowering, each plant in the 24 "injury" pots was pinched twice at the base of each stem supporting a flower, with a self-closing clothespin. This procedure corresponded to that used by Franzke and Hume (6) in their experiments on Sorghum, and was intended to simulate mechanical injury such as might result in many ways during the growing season, and during harvesting.

At maturity the seeds were collected and cleaned. Just before the cyanide determinations were carried out, the seeds were ground in a glass mortar. Where possible, two assays were made on the yield from each pot. To the ground sample, in the tube of a Van Slyke – Cullen Aeration Apparatus (7), was added a trace of granular stearic acid to prevent frothing, and 10 ml. of distilled water. After 15 min. maceration (the sample tube of the apparatus being suspended in a thermostat maintained at 45°C.), air was passed through the mixture at a slow steady rate for two hours. The 45° temperature maintained was the optimum temperature for enzymatic hydrolysis of the cyanogenetic glycoside linamarin to its decomposition products, D-glucose, acetone, and hydrogen cyanide. An alkaline trap (10 ml. of $N/10$ aqueous sodium hydroxide) completely removed the hydrogen cyanide from the air circulated through the sample. Five milliliters of concentrated ammonium hydroxide and 2 ml. of 5% aqueous potassium iodide were added to the contents of the trap, and the estimation of the cyanide was carried out with $N/800$ aqueous silver nitrate, according to the method of Liebig, as modified by Sharwood (14).

Attempts were made to find a test paper suitable for the roughly quantitative estimation of potential cyanide in flax. Among the tests tried were the copper acetate – benzidine acetate (5), the copper sulphate – guaiacum (5), the phenolphthalin (15) and the alkaline picrate method (1), the last-named despite the fact that acetone, a decomposition product of linamarin, normally reacts with the reagent (10). In each case, a small piece (5 by 60 mm.) of filter paper, impregnated with the specific reagent, was suspended in a corked 10 by 75 mm. test tube above 0.025 to 0.100 gm. of finely ground flax, moistened with distilled water and left at room temperature.

Results and Discussion

The results obtained from the analyses of the samples are listed in Table I.

TABLE I

MEAN HYDROGEN CYANIDE CONTENT OF ROYAL AND REDWING FLAX FOR EIGHT TREATMENTS

Moisture level	Condition	Royal		Redwing	
		No. of samples	Mean mgm. % HCN	No. of samples	Mean mgm. % HCN
Low	Control	11	26.4	11	33.7
Low	Frost	4	47.2	11	57.9
Low	Injury	11	29.0	11	34.7
Low	Drought	8	26.7	9	36.1
High	Control	11	19.6	12	23.0
High	Frost	7	47.7	12	49.6
High	Injury	10	24.5	11	26.8
High	Drought	9	26.8	11	28.1
		71	28.9	88	36.2

Of the 192 theoretically possible samples, 33 (17.2%) were lost owing to (*a*) plant fatality due to frost or injury, (*b*) poor yield due to adverse effects created by the various treatments, and (*c*) laboratory accidents. The average cyanogenetic content of the 159 samples was 32.9 mgm. % of hydrogen cyanide. Table I illustrates that in all comparable treatments Redwing contains more potential cyanide than Royal. Table III shows that this constant difference is highly significant.

The usual method of analysis of variance could not be carried out for these data owing to the irregularity of numbers of samples for the different treatments. Therefore, the Yates' method of unweighted means for the disproportionate subclass numbers (assuming interaction present) was used for the present analysis (16). The analysis of variance is shown in Table II. *F* values show that the effects of variety and of soil moisture level are highly significant.

TABLE II

ANALYSIS OF VARIANCE TO DETERMINE EFFECT OF VARIETY, MOISTURE, FROST, INJURY, AND DROUGHT ON CYANOGENETIC CONTENT OF FLAX

Sources of variation due to	*F*
Varieties	412.44**
Moisture	247.12**
Treatments	934.18**
$V \times M$	96.66**
$V \times T$	1.43
$M \times T$	6.84**
$V \times M \times T$	43.90**

** *Significant beyond the 1% point.*

The total effect of the treatments is also very great. The secondary interactions, in two of three cases, are highly significant. The soil moisture level has a differential effect on the two varieties as well as on the conditions imposed. However, variety does not in any way alter the effect of the treatments applied. The tertiary interaction is highly significant, doubtless owing to the two significant secondary interactions.

Table III is introduced to illustrate the effect of the individual conditions, as well as to confirm the significance of variety and moisture. Flax grown in soil of lower moisture content contains significantly more linamarin than flax grown with access to more soil moisture. Redwing flax contains significantly more potential cyanide than does Royal. While frost more than doubles the amount of cyanogenetic glycoside in flax, injury and drought merely increase

TABLE III

SUMMARY OF EFFECTS OF VARIETY, MOISTURE, AND CONDITION (FROST, INJURY, DROUGHT) ON THE CYANOGENETIC CONTENT OF FLAX

Classification	Mean, mgm. % HCN	$d \pm S.E._d$
Moisture		
Low moisture	35.9	5.6 ± 0.35**
High moisture	30.3	
Variety		
Royal	28.9	
Redwing	36.2	7.3 ± 0.36**
Condition		
Control	25.6	
Frost	51.6	26.0 ± 0.51**
Injury	28.8	3.2 ± 0.48**
Drought	29.4	3.8 ± 0.49**

** Significant beyond the 1% point.

the quantity by an average of 13% and 17% respectively. However, the action of all three climatic factors is significant at the 1% level, as shown in Table III. Fig. 1 illustrates all these effects graphically.

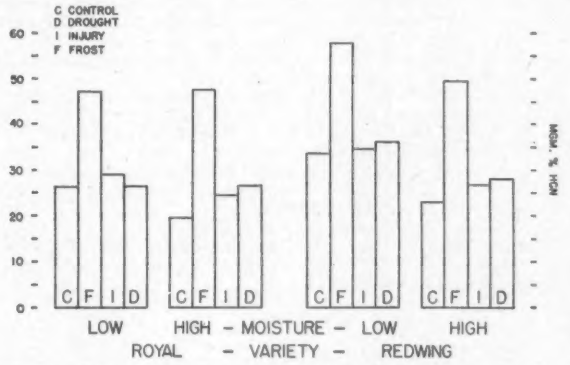

FIG. 1. *Effect of some factors on hydrogen cyanide content of flax.*

Acetone normally reacts with the alkaline picrate reagent in the same way as hydrogen cyanide. A positive test for acetone in the alkaline trap was obtained by means of the iodoform reaction. However, $M/50$ and $M/100$ aqueous solutions of acetone had little or no effect on alkaline picrate paper suspended above them for 24 hr. Under similar conditions, $M/50$ and $M/100$ solutions of hydrogen cyanide, and a solution $M/100$ with respect to both, reacted almost immediately with the reagent, reaching maximum intensity of color in 20 to 30 min. The last (with acetone added) showed no deeper color than did

its control. On the other hand, test paper suspended above ground flax moistened with water and containing 0.031% hydrogen cyanide did not begin to change color for 90 min. and reached maximum color in six to eight hours. This delay in color formation was doubtless due to slow hydrolysis at room temperature. It seems likely that alkaline picrate test paper may be used for the preliminary estimation of potential cyanide in flax. The test appears able to detect differences of about 10 mgm. %.

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